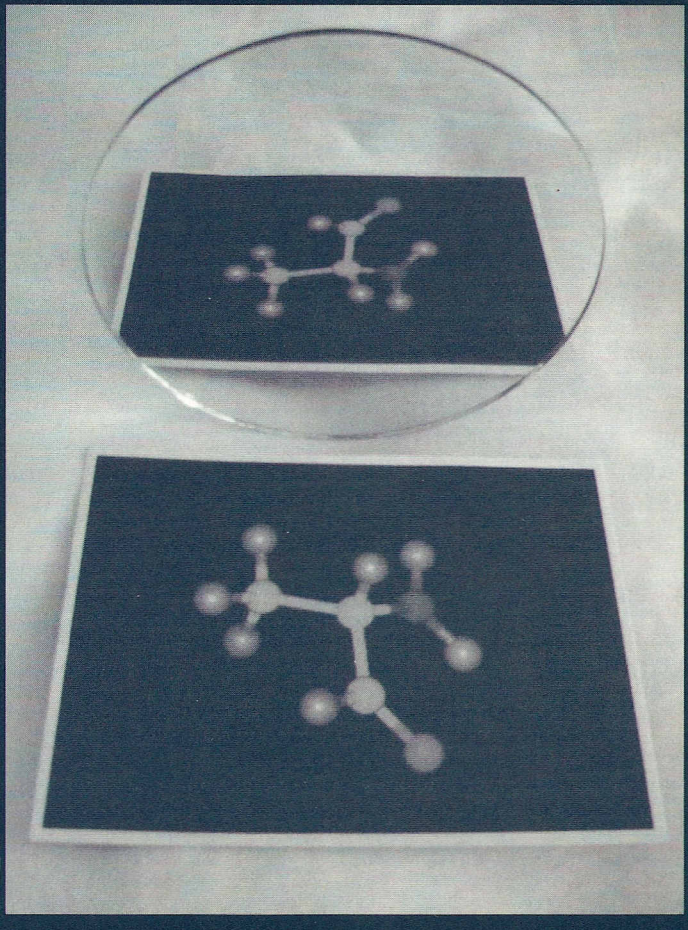
Geologica Ultraiectina

Mededelingen van de
Faculteit Aardwetenschappen
Universiteit Utrecht
No. 213

Early diagenesis of amino acids in NE Atlantic continental margin sediments



Mark M.C.H. Grutters

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Early diagenesis of amino acids in NE Atlantic continental margin sediments.

Vroege diagenese van aminozuren in sedimenten van de Noordoostelijke Atlantische continentale helling.
(Mat een samenvatting in het Nederlands)

PROEFSCHRIFT

Ter verkrijging van de graad van doctor
aan de Universiteit Utrecht,
op gezag van de Rector Magnificus Prof. dr. W. H. Gispen
ingevolge het besluit van het College voor Promoties
in het openbaar te verdedigen
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door

Mark Marinus Cornelis Hendrikus Grutters
Geboren op 10 januari 1972, te Boxmeer

Promotor: Prof. Dr. J.W. de Leeuw
Co-promotor: Dr. W. van Raaphorst

Contents

Voorwoord

Hier is het resultaat van vier jaar wetenschappelijk onderzoek-in-opleiding. Een periode die niet altijd even gemakkelijk is geweest, maar wel uiterst boeiend en leerzaam. Van tijd tot tijd heb ik geprobeerd wat afstand te nemen van mijn onderzoek en dan eens goed na te denken over de ontwikkelingen die ik als promovendus heb doorgemaakt; langzaam veranderend van een tamelijk naieve en ongeduldige academicus in een kritische en meer bedaarde wetenschapper. De metamorfose van iemand die meent dat hij alles weet tot iemend die zich ervan bewust is dat hij juist helemaal niet veel weet! In het laatste stadium van mijn onderzoek ging ik inzien dat eigenlijk iedere wetenschapper veel niet weet en dat mijn bijdrage aan de wetenschap wel degelijk hiaten vult. Dat geeft veel voldoening. Dat is vier jaar hard werken aan je onderzoek ruimschoots waard!

Het NIOZ is een fantastische plaats voor wetenschappelijk onderzoek-in-opleiding. Vooral het multidisciplinaire en multinationale karakter van het NIOZ biedt een prachtige kans om met veel verschillende mensen vanuit verschillende standpunten van gedachten te wisselen over je onderzoek.

In de eerste plaats wil ik alle mensen van het NIOZ bedanken bij wie ik de laatste 4 jaren ben binnengelopen met de meest uiteenlopende vragen en ideeën. Uiteindelijk bleken de meeste ideeën bij voorbaat totaal onuitvoerbaar, of niet succesvol na een periode van experimenteren. Maar wanhoop niet! Alle tijd die jullie aan me hebben verknoeid was toch leerzaam en waardevol. Ik heb vooral geleerd hoe je experimenten NIET moet uitvoeren!

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Bas and Jerome. I would like to thank you for being my colleagues and especially my good friends. Our coffee sessions after lunch were always very 'interesting'. I think that people who have seen our 'hot' discussions must have thought that we were fighting. I really enjoyed our exhausting squash games. After all, people say "anima sana in corpore sano". I also appreciated the work you did for me as my 'paranimfen'. You have been a great help for me when I was in the United States.

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Iedereen kent tegenslagen. Zo zette de dood van mijn broer tijdens mijn onderzoek me weer met twee benen op de grond. Je denkt na over wat er echt belangrijk is in het leven. Is het echt nodig om zo hard te werken om per se dat onderzoek in vier jaar af te ronden? Ik was van plan mijn tijd beter te besteden en te gaan genieten van iedere dag. En tot mijn grote schrik realiseerde ik me enkele maanden later dat je ongemerkt weer bent afgegleden naar het oude patroon van haasten, druk en te hard werken. Ik probeer vaak te denken aan hoe ik me toen voelde en welke afspraak ik met mezelf had gemaakt. Dat helpt, voor een korte tijd...

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Ik wil graag eindigen met een citaat van mijn grote voorbeeld, professor Prlwytzkofski. Hij kan als geen ander fenomenen abstraheren tot in hun essentie en is volkomen objectief. Eigenschappen die elke goede wetenschapper zou moeten bezitten.

> *"Professor Prlwytzkofski haalde enkele sterke vergrootglazen uit zijn koffertje en hurkte op de grond neer om de vreemde bolletjes te bekijken.*
> *'Praw!' mompelde hij na enkele ogenblikken. 'Zij zien er uit als gans gewoonlijke oogballen, mijnheer Pieps. Onschadelijk, zou men zeggen. Maar betracht de eigendommelijke straling des pupils, bid ik u. Er verschuilt zich een sterke aard energie in deze fenomenen!'*
> *'Zou-zouden ze kwaad kunnen?' vroeg de assistent met bevende stem.*
> *'Dat moet blijken,' hernam de geleerde. 'Voor mij staat vast, dat wij ons tegenover protoplast bevinden, dat een vorm heeft aangenomen. En der vorm...'*
> *Zijn stem stierf weg toen de vreemde balletjes zijn jas beklommen en geruime tijd sloeg hij ze oplettend gade.*
> *'Ze zijn zeer aanhankelijk,' prevelde hij toen. 'Of nieuwsgierig. Zij beelden ons na; zij onderzoeken ons.'*
> *'Ik vind het maar eng,' riep Alexander Pieps uit. De hoogleraar wilde hem op het onwetenschappelijke van deze benadering wijzen; maar op dat moment werd zijn aandacht getrokken door een rimpeling in de oppervlakte van het meer. Er verschenen enige ronde vormen boven de stroperige vloeistof, die zich snel naar de kust bewogen. En toen klommen er een aantal eigenaardige figuurtjes aan land, tot grote verassing van de geleerden.*
> *'Nah!' riep professor Prlwytzkofski. 'Der plasma neemt andere vormen aan! Die zien ja gans drollig uit!'*
>
> Uit Marten Toonder. *De kwade inblazingen* 1967

Samenvatting

In dit proefschrift beschrijf ik de *vroege diagenese* van aminozuren in sedimenten van de NO Atlantische continentale helling. De vroege diagenese omvat de afbraak- en transformatieprocessen ('veroudering') die plaatsvinden voorafgaand aan de depositie van organisch materiaal en tijdens het verblijf in de bovenste lagen van het sediment.

Aminozuren, de bouwstenen van eiwitten, worden door algen geproduceerd in de oppervlakte van de oceaan tijdens fotosynthese. Een deel van de aminozuren zal vanuit de oppervlakte van de oceaan (de eufotische zone) naar de diepzee worden getransporteerd in de vorm van grote, snelzinkende aggregaten. Stikstofrijke verbindingen, zoals aminozuren, worden sneller afgebroken dan stikstofarme verbindingen, zoals bijvoorbeeld vetten. Daarom zal de bijdrage van aminozuren aan de totale hoeveelheid organisch materiaal afnemen met de ouderdom van het organisch materiaal, en dus met de toenemende diepte in de waterkolom. Bovendien treden er in het organisch materiaal verschuivingen op in de verhouding tussen de individuele aminozuren, door bijvoorbeeld verschillen in de voedingswaarde van aminozuren, verschillen in adsorptiecapaciteit en/of weerstand tegen bacteriële afbraak.

Het bestuderen van de concentratie van totaal hydrolyseerbare aminozuren (THAA) in monsters van zowel sedimentvallen als van de zeebodem toont aan dat aminozuren uitstekend geschikt zijn om de diagenetische 'veroudering' vast te stellen van organisch materiaal langs de Goban Spur continentale helling (NO Atlantische Oceaan). Zowel de lage bijdrage van aminozuren aan het totaal organisch materiaal als de geringe verschuivingen in de verhouding tussen de individuele aminozuren wijzen erop dat het organisch materiaal al substantieel is afgebroken bij afzetting op het sediment oppervlak. Een diagenetisch model is toegepast op de gemeten concentratieprofielen van THAA en totaal organisch koolstof in de sedimenten om hun afzetting op het sediment oppervlak, de menging in het sediment en hun afbraak te berekenen. De resultaten geven aan dat THAA alleen boven op de continentale helling sneller worden afgebroken dan totaal koolstof. Dat bevestigt dat het organisch materiaal op de lager gelegen helling een 'ouder' karakter heeft. De diagenetische 'veroudering' van het organisch materiaal in de sedimenten langs de helling van de Goban Spur komt beter tot uiting door het bestuderen van korrelgroottefracties van de toplaag van het sediment. Verschuivingen in de verhouding tussen de aminozuren in de korrelgroottefracties tonen aan dat het organisch materiaal in de fijnste fractie (<0.5 μm) boven aan de helling een veel 'jonger' karakter heeft dan in de grovere fracties en 'jonger' dan alle korrelgroottefracties in sedimenten beneden aan de helling. De bijdrage van fijne deeltjes neemt toe met de diepte langs de helling en dat suggereert dat deze fijne deeltjes eroderen op de bovenhelling en accumuleren op de benedenhelling. Dit hellingafwaarts transport van fijne deeltjes, gecombineerd met de continue afbraak van organisch materiaal dat is geadsorbeerd aan deze deeltjes, heeft tot gevolg dat de diagenetische 'veroudering' van het organisch materiaal toeneemt met de diepte langs de continentale helling.

Langs de helling van het Faeroer-Shetlands Kanaal (FSC) in de NO Atlantische Oceaan is het hellingafwaartse sediment transport, door zich herhalende cycli van erosie-depositie, een veel belangrijker mechanisme voor de afzetting van organisch materiaal in de diepzee dan verticaal transport vanuit de eufotische laag van de oceaan. Er is een

compositiemodel beschreven dat is gebaseerd op zowel totaal stikstof (TN), THAA als op de samenstelling van individuele aminozuren in monsters van gesuspendeerd materiaal (SM) uit de waterkolom, van sedimentvallen en van het sediment. Het model toont aan dat het SM dicht bij de zeebodem voor ~80% bestaat uit fijne deeltjes (<63 μm) afkomstig van het sediment oppervlak. Deze fijne deeltjes blijken semi-permanent in suspensie te zijn. De bijdrage van de verticale aanvoer uit de waterkolom is ~20%. De bijdrage van aminozuur-stikstof aan TN in SM neemt toe met de waterdiepte, en dat ondersteunt de model resultaten dat slechts een klein deel van het organisch materiaal dicht bij de zeebodem afkomstig is van de eufotische zone van de oceaan. Asparaginezuur is zowel verrijkt in zowel SM dicht bij de zeebodem en in het sediment boven aan de continentale helling als in monsters uit de sedimentvallen op de lager gelegen helling. Dit suggereert dat aminozuren, geassocieerd met carbonaten, uit het sediment boven aan de helling in suspensie worden gebracht en langs de helling naar beneden worden getransporteerd. Het maximum van fijne deeltjes halverwege de helling (750 m diepte), de verhoogde bijdrage van aminozuur-stikstof aan TN en de verhoogde aanvoer van het radioactieve ^{234}Th op de deze diepte bevestigen dat jong, aminozuurrijk organisch materiaal hellingafwaarts wordt getransporteerd en hoofdzakelijk wordt afgezet op 750 m diepte.

Een deel van de aminozuren die worden afgebroken door bacteriën in de waterkolom en in het sediment worden gebruikt voor de synthese van nieuwe biomassa. Aminozuur-enantiomeren zijn in dit proefschrift gebruikt om de bijdrage van aminozuren uit de bacteriële synthese aan THAA in het sediment te identificeren. Aminozuren in eiwitten zijn gewoonlijk opgebouwd uit L-aminozuren. Gepolariseerd licht dat door deze L-aminozuren (*Levorotary*) valt zal tegen de klok in worden geroteerd; ze worden dus ook wel linksdraaiend genoemd. Deze L-aminozuren kunnen door een chemisch proces (racemizatie) worden omgezet in D-aminozuren (*Dextrorotary*; rechtsdraaiend), welke exact dezelfde chemische eigenschappen bezitten maar een spiegelbeeld zijn van de L-aminozuren. Op een tijdschaal van enkele honderdduizenden tot miljoenen jaren zullen in de restanten van eiwitten in organisch materiaal evenveel L- als D-aminozuren aanwezig zijn. Echter, bacteriën zijn een van de weinige soorten organismen die naast L- ook D-aminozuren produceren voor peptidoglycan, een belangrijk onderdeel van hun celwand. Dus zodra D-aminozuren worden gedetecteerd in monsters van jonge leeftijd is het zeer aannemelijk dat deze van bacteriën afkomstig zijn.

Langs de Goban Spur continentale helling was de bijdrage van racemizatie aan de gemeten hoeveelheid D-aminozuren in het sediment verwaarloosbaar en dat wijst dus op een bacteriële bron van D-aminozuren. Deze D-aminozuren zijn waarschijnlijk niet geassocieerd met hele cellen, omdat uit berekeningen blijkt dat de totale hoeveelheid aminozuren die hiermee geassocieerd zou zijn (D & L) de gemeten concentratie aminozuren in de monsters met een factor 5 zou overschrijden. DL-aminozuren die geassocieerd zijn met alleen celwanden (dus resten van afgestorven bacteriën) vormen ~10% van THAA in het sediment oppervlak en dieper in het sediment zelfs meer dan een derde van THAA. Een diagenetisch model is toegepast op de THAA concentratieprofielen die zijn gecorrigeerd voor de bijdrage van aminozuren uit bacteriecelwanden. De afbraakconstanten van aminozuren, berekend met dit model, zijn 2-10 keer hoger dan de afbraakconstanten die zijn berekend uit de oorspronkelijke (niet gecorrigeerde) profielen. Dit bewijst dat het niet betrouwbaar is om de afbraaksnelheden te berekenen van aminozuren die uit de waterkolom

worden afgezet op het sediment als er geen rekening wordt gehouden met de synthese van aminozuren door bacteriën.

Een compositiemodel gebaseerd op D-aminozuren in gesuspendeerd materiaal in de water kolom, sedimentvallen en sedimenten, toont aan dat het erosie-depositie mechanisme dat verantwoordelijk is voor het transport van THAA langs de helling van het FSC ook verantwoordelijk is voor het transport van D-aminozuren. D-aminozuren in gesuspendeerd materiaal dicht bij de zeebodem zijn niet afkomstig uit de eufotische zone van de oceaan, maar van fijne deeltjes die opwervelen van het sediment oppervlak. De bijdrage van D-aminozuren uit racemizatie aan THAA in de sedimenten langs de helling van het FSC bedraagt gemiddeld ~5%. De hoeveelheid D- en L-aminozuren in de bovenste 5 cm van het sediment, per cm^2 sedimentoppervlak, vertoont een duidelijk maximum op 750 m diepte. De ratio tussen D- en L-aminozuren uit deze hoeveelheden per cm^2 neemt af met de diepte langs de helling van het FSC. Dit suggereert dat de bijdrage van (nieuw gesynthetiseerde) hele bacteriecellen toeneemt met de diepte langs de helling. Deze resultaten leiden tot de conclusie dat het hellingafwaarts transport van fijne, organisch materiaalrijke deeltjes de toename van bacteriën initieert, waarbij aminozuren worden gebruikt om bacteriecelwanden te vormen. Dit resulteert in een accumulatie van resistente bacteriecelwanden op 750 m diepte. In het sediment worden de (resten van) de bacteriecelwanden gepreserveerd. De bijdrage van DL-aminozuren uit deze celwanden aan THAA bedraagt ten minste ~24% in de bovenste 5 cm van het sediment.

Een belangrijke implicatie van de bacteriële synthese van aminozuren is dat afbreekbare aminozuren worden omgezet in labiel celplasma en relatief resistent celwand materiaal. Deze omzetting vergroot de hoeveelheid aminozuren uit de eufotische zone van de oceaan dat de vroege diagenese 'overleeft'. Dit vormt mogelijk een eerste stap in de lange-termijn preservatie van aminozuren, en dus ook van totaal organisch materiaal, in sedimenten langs de NO Atlantische continentale helling.

Summary

This thesis concentrates on *early diagenesis* of amino acids in sediments across the NE Atlantic continental slope. Early diagenesis comprises the degradation and transformation processes that take place during transport of amino acids through the water column and the early stages of burial in the sediments.

Amino acids, constituents of proteins, are generally transported through the water column by large, rapidly sinking aggregates. During this transport, nitrogen-rich compounds like amino acids are degraded faster than nitrogen-poor compounds (e.g. lipids). Therefore, the contribution of amino acids to bulk organic matter decreases with ageing of the organic matter, and hence with increasing depth in the water column. Moreover, shifts occur in amino acid distributions due to differences in nutritional value, adsorption capacity, resistance against degradation, etc.

A study on total hydrolysable amino acids (THAA) in sediment trap samples and in sediments across the Goban Spur continental slope (NE Atlantic) demonstrated that amino acids can be used to assess the diagenetic state of organic matter in the sediments. The very low contribution of THAA to bulk organic matter as well as the rather constant amino acid distributions in bulk sediments indicated that organic matter was already substantially degraded prior to incorporation into the sediments. A diagenetic model was applied to measured THAA and total organic carbon concentration profiles in the sediments to study their inpiut, mixing and degradation. Amino acids were degraded faster than total organic carbon at the upper slope only, confirming the relatively refractory character of the organic matter in the sediments at the lower slope. The difference in diagenetic state of the organic matter across the slope became clear by studying size fractions of the sediment top layer. Shifts in amino acid distributions indicated that the organic matter in the finest fraction (<0.5 μm) was more labile than that in coarser fractions at the upper slope and than any size fraction at the lower slope. The contribution of fine particles increased with depth across the slope suggesting they were eroded from the upper slope and accumulated at the lower slope. From this down-slope transport in combination with the continuous degradation of organic matter attached to the fine particles it was concluded that the organic matter degradability decreased from the upper slope to the deep-sea.

Across the slope of the Faeroe-Shetland Channel (FSC) in the NE Atlantic, down-slope particle transport by repetitive cycles of erosion-deposition was far more important for the delivery of organic matter to deep-sea sediments than vertical settling from the euphotic layer of the ocean. An end-member model based on total nitrogen (TN) and THAA concentrations as well as on amino acid distributions in suspended matter (SM) samples from the water column, sediment trap samples and surface sediments demonstrated that near-bottom SM comprised ~80% of fine particles that were resuspended from the sediment surface. The vertical flux from the upper water column contributed only to ~20%. The contribution of amino acid-N to TN in suspended matter increased with water depth and supported the model results that organic matter in near-bottom water was not derived from the euphotic layer. The enrichment in aspartic acid in near-bottom SM and sediments at the upper slope as well as in sediment trap samples at the lower slope suggested that carbonaceous particles were eroded from the upper slope and transported down in near-bottom SM layers. The mid-slope maximum of fine particles in the surface sediment, the

elevated inventories of ^{234}Th as well as the high contribution of amino acid-N to TN evidenced that young and amino acid-rich organic matter was transported down-slope and preferentially settled at mid-slope depth.

Part of the amino acids that are degraded by bacteria in the water column and in the sediments are used for synthesis of new biomass. Amino acid enantiomers were used to identify the contribution of bacterially derived amino acids to THAA in the sediments. Amino acids in proteins are generally composed of L-amino acids, which can a-biotically racemize to D-amino acids, the mirror image of the L-enantiomer, on time scales of a few hundred thousands to millions of years. Bacteria, however, are among the few organisms that can produce D-amino acids for peptidoglycan, the main constituents of their cell walls.

Across the Goban Spur, the contribution of D-amino acids by racemization was almost negligible which pointed at a bacterially source for D-amino acids. D-amino acids were likely not associated with whole bacterial cells, since the contribution of amino acids from whole cells would exceed measured THAA concentrations in the sediments by a factor 5. Amino acids from bacterial cell wall remnants could account for ~10% of THAA in the sediment surface and for more than one third of THAA in the deeper sediments. A diagenetic model was applied to THAA concentration profiles lowered for bacterially derived amino acids. First-order degradation rate constants of THAA were 2-10 times higher than rate constants calculated from original profiles, clearly indicating that studying THAA degradation without taking into account the synthesis of amino acids by bacteria in the sediments is unreliable.

An end-member model based on D-amino acids in suspended matter from the water column, sediment trap samples and surface sediments demonstrated that the erosion-deposition mechanism that was responsible for the down-slope transport of THAA across the FSC was also responsible for the distribution of amino acid enantiomers. D-amino acids in near-bottom water layers appeared not to be derived from the euphotic layer but from fine particles resuspended from the sediment surface. On average, approximately 5% of the D-amino acid concentration in the sediments could be accounted for by a-biotic racemization. Inventories of D and L-amino acids showed that there was a pronounced mid-slope accumulation of both enantiomers in the upper 5 cm of the sediments relative to the other stations. The ratio of D/L-amino acids in the upper sediments, obtained from the inventories, decreased with depth across the slope and indicated that the contribution of (newly synthesized) whole cells may increase down-slope. It was concluded that bacterial growth on fine, labile organic matter-rich particles transported down-slope in near-bottom water layers resulted in a mid-slope accumulation of D-amino acids in refractory bacterial cell wall material. With increasing depth in the sediments these amino acids are preserved as cell wall remnants and contributed at least 24% to THAA in the deeper sediments.

An important implication of the bacterial synthesis of amino acids is that degradable amino acids are transformed into labile cell plasma and relatively refractory bacterial cell walls. This conversion into cell wall material may enlarge the proportion of amino acids from primary production that survives early diagenesis, and could be the first step in the long-term burial of amino acids in marine sediments.

1.

Introduction and outline

Early diagenesis of organic carbon in marine sediments

Of the earth's carbon more than 99% is stored in sedimentary rocks (~70 10^{21} g C) and is withdrawn from the global carbon cycle for millions of years (Kempe, 1979). Of the less than 1% of carbon that actively participates in the carbon cycle, dissolved inorganic carbon (DIC) in the ocean is by far the largest reservoir (~36 10^{18} g C; Murray, 1992). From this DIC, which includes carbon dioxide (CO_2), approximately ~0.6 10^{18} g particulate organic carbon (POC) is annually produced by photosynthesis (Romankevich et al., 1999). Phytoplankton accounts for more than 90% of organic matter (comprising the POC) production in the ocean. Although the majority of the organic matter is degraded by zooplankton and bacteria and returned into CO_2 and nutrients available for new phytoplankton growth, a small part escapes from the euphotic zone and is transported through the water column by large, rapidly sinking (~100-200 m d^{-1}) aggregates (Honjo et al., 1982a; Fowler and Knauer, 1986). These aggregates are rich in easily degradable (labile) compounds as carbohydrates and proteins and are, therefore, active sites of microbial degradation (Cho and Azam, 1988; Alldredge and Gotschalk, 1990). The amount of organic matter that reaches the seafloor and finally becomes buried in the sediments is linked to the primary production in the ocean's surface (Pedersen and Calvert, 1990; Calvert and Pedersen, 1992) and/or to the rate by which the organic matter is deposited (Henrichs and Reeburgh, 1987b). In shelf seas with high primary productivity as much as 20-50% of the organic matter might reach the sediments (Jørgensen, 1983), whereas in the open ocean generally 1-3% reaches the seafloor (Suess, 1980; Lampitt and Antia, 1997; Lee et al., 1998; Jahnke, 1996; Romankevich et al., 1999) due to the longer travelling and, hence, degradation time in the water column.

Near continental slopes, total mass fluxes as well as fluxes of POC can increase several-fold with water depth (Honjo, 1982b; Antia et al., 1999; Biscaye and Anderson, 1994). This increase close to the sea floor is not directly related to POC production in the overlying water column but originates from resuspension of particles from the seafloor. These layers of resuspended particles (nepheloid layers) are particularly formed at the continental shelf-break, from where they can protrude laterally into the interior of the ocean (Dickson and McCave, 1986) or follow the continental slope in bottom nepheloid layers (BNL) (McCave, 1986). The intensity of these BNL depend on the strength of the bottom currents (McCave, 1986) and the sediment bed structure (Thomsen, 1999). At continental slopes with strong currents, down slope particle transport may importantly contribute to the deposition of POC at deep-sea sediments in addition to vertical settling of POC from the surface.

Early diagenesis comprises the degradation and transformation processes that take place prior to deposition of the POC at the sediment surface and upon the early stages of burial. During early diagenesis ~90% of the POC becomes degraded by bacteria and benthic organisms and ultimately less than 1% of the POC produced by photosynthesis (0.02-0.2 10^{15} g C) becomes buried in the ocean sediments (Berner, 1982; Romankevich et al., 1999).

Although continental margins (shelf and slope) comprise only ~11% of the global ocean surface (Sverdrup et al., 1946), they account for more than 80% of POC deposition and burial (Müller and Suess, 1977; Berner, 1982; Romankevich et al., 1999). However, continental margins include a wide range of environmental conditions that influence the early diagenesis and, hence, determine the amount of POC ultimately buried. For example, repetitive cycles of resuspension-deposition and disaggregation-aggregation in the BNL hydrodynamically sort particles and results in a down slope increase in small POC-rich particles (Thomsen and Van Weering, 1998; Thomsen, 1999). Preferential adsorption of POC to mineral surfaces of small particles, due to their large specific surface areas (Mayer, 1994a, 1994b), potentially protects intrinsically labile POC from microbial degradation (Keil et al., 1994b). By contrast, the repeated disaggregation-aggregation and transport of POC-rich particles in the BNL may stimulate the growth of deep-sea bacteria and, hence, the degradation of POC (Turley and Lochte, 1990; Ritzrau, 1996; Boetius et al., 2000a; Nagata et al., 2000). In the sediments, POC degradation is influenced by the length of time it is exposed to oxygen (Hedges and Keil, 1995; Hartnett et al., 1998). Although the microbial degradation of labile organic matter constituents (carbohydrates, proteins) is similar under conditions with and without oxygen (Westrich and Berner, 1984; Lee, 1992; Kristensen et al., 1995; Thunell et al., 2000), more complex organic matter molecules are degraded efficiently under aerobic conditions only (Harvey et al., 1995; Sun et al., 1997). Benthic macro- and meiofauna, whose distribution is influenced by sediment resuspension and deposition, utilise POC but may also stimulate the microbial POC degradation since they remove inhibitory metabolites and transfer fresh POC from the sediment surface to the deeper layers by burrowing and sediment mixing (Aller, 1982; Aller and Yingst, 1985; Aller, 1989). Moreover, re-exposure of POC from anoxic sediment layers to oxygen by sediment mixing results in a more complete degradation than under constant anoxic or oxic conditions (Aller, 1994).

Amino acids as indicators for the diagenetic state of organic matter

Although many processes that explain early diagenesis of POC have been proposed, none of these are fully conclusive and some may even have contrasting results. Amino acids, constituents of proteins, have often been used as a representative class of compounds to study early diagenesis of labile organic matter. With respect to total organic matter, nitrogen-rich constituents such as amino acids are preferentially degraded. As a result, the contribution of amino acid nitrogen to total nitrogen (%-AAN) decreases from 75-90% in fresh plankton to 40-50% in sinking aggregates and finally to 10-30% in sedimentary organic matter (Lee, 1988 and references therein). Cowie and Hedges (1992b) used the %-AAN as an indicator of the diagenetic state of the organic matter and they showed that values of %-AAN below 38% were indicative of severely degraded organic matter.

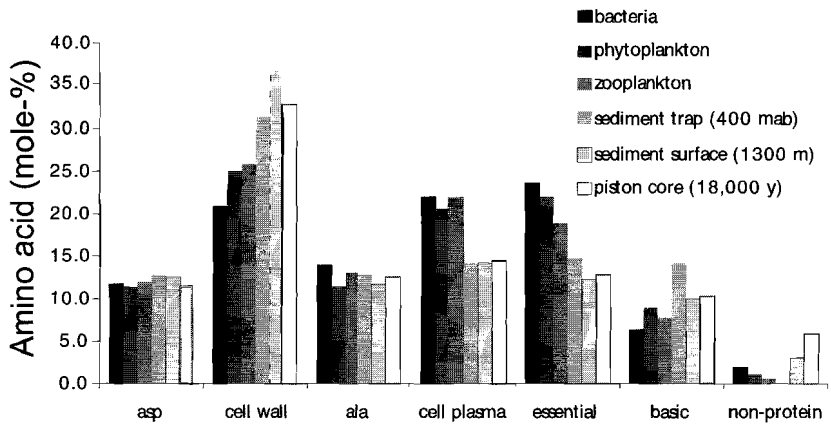

Figure 1.1 Mole-percentages of the amino acids as measured by HPLC in samples from labile organic matter end-members as bacteria, phytoplankton and zooplankton (Brown, 1991; Cowie and Hedges, 1992b; Dauwe and Middelburg, 1998) and from more refractory organic matter of sediment traps, surface sediments and piston cores (representing an organic matter age of ~18,000 y; Grutters et al., 2001a). Here, amino acid classes are shown rather than individual amino acids. ASP denotes aspartic acid; CELL WALL denotes glycine, serine and threonine; ALA denotes alanine; CELL PLASMA denotes glutanmic acid, tyrosine and phenylalanine; ESSENTIAL denotes methionine, valine, iso-leucine and leucine; BASIC denotes lysine, arginine and histidine; NON-PROTEIN denotes β-alanine and γ-aminobutyric acid.

Approximately 20 amino acids are commonly found in proteins and they can be measured accurately by high performance liquid chromatography (HPLC). Several studies illustrated that the relative contribution of the individual amino acids to the total hydrolysable amino acid (THAA) pool changes during early diagenesis. Some of the neutral amino acids (valine, methionine, phenylalanine, isoleucine, leucine) cannot be synthesised from simple precursors by most organisms but they must be obtained from their diet. Therefore, these so-called essential amino acids become relatively depleted during early diagenesis (Philips, 1984; Cowie and Hedges, 1992b; Dauwe and Middelburg, 1998) (Fig. 1.1). Glutamic acid, tyrosine and phenylalanine are associated with phytoplankton cell plasma, and these relatively labile amino acids have often been found to decrease during early diagenesis (Hecky et al., 1973; Cowie and Hedges, 1992b; Cowie et al., 1992c). Glycine, serine and threonine, usually associated with diatoms cell walls (Hecky et al., 1973), generally become enriched during early diagenesis due to the large contribution of diatoms to primary production, their high sinking rates and the relative stability of diatom cell walls (Burdige and Martens, 1988; Cowie et al., 1992c; Cowie and Hedges, 1992b). Non-protein amino acids (β-alanine and γ-aminobutyric acid) are present in marine organisms in small quantities only, and their relative contribution increases during early diagenesis as a result of the decarboxylation of their precursor acidic amino acids (Lee and Cronin, 1982). Basic amino acids (lysine, histidine, arginine) are positively charged at the pH of ocean water and adsorb relatively easily to negatively charged clay mineral surfaces (Hedges and Hare, 1987; Henrichs and Sugai, 1993; Wang and Lee, 1993). They are, therefore, assumed to be less susceptible to microbial degradation (Keil et al., 1994b; Schuster et al., 1998; Hulthe et

al., 1998). Acidic amino acids (aspartic and glutamic acid) are negatively charged at the pH of ocean water and preferentially adsorb to positively charged calcium carbonate particles (Müller and Suess, 1977).

A Principal Component Analysis (PCA) can be applied to amino acid distributions in marine samples from different environmental conditions to further evaluate the early diagenesis of labile organic matter. A PCA statistically detects correlations among measured variables. Variables that are correlated with each other, but that are independent of other variables, are combined into principal components (PC). These principal components may be interpreted as indicators of the underlying processes that caused the correlations among the variables. Fig. 1.2 exemplifies the results of a PCA for the amino acid classes shown in Fig. 1.1. The ESSENTIAL, CELL PLASMA and CELL WALL amino acids are correlated and they combine to one PC. This first Principal Component (PC) reflects the ageing of organic matter, with relatively high contributions of essential and cell plasma amino acids in labile organic matter (negative site of PC1) and increasing contributions of cell wall amino acids in more refractory organic matter (postive site of PC1). The first PC can thus be interpreted as a degradation index, as was introduced by Dauwe and Middelburg (1998). According to their position along the first PC, samples from bacteria, phytoplankton and zooplankton are younger, and less degraded than samples from sediment traps and sediments. Both ASP and NON-PROTEIN appear rather independent of the first PC and combine to a second PC. The position of the samples along the second PC may indicate that the sediment trap sample is enriched in aspartic acid, and going from the water column (trap) to the sediments (piston core) aspartic acid is transformed into its non-protein derivative. The second PC may be interpreted as an index for microbial degradation.

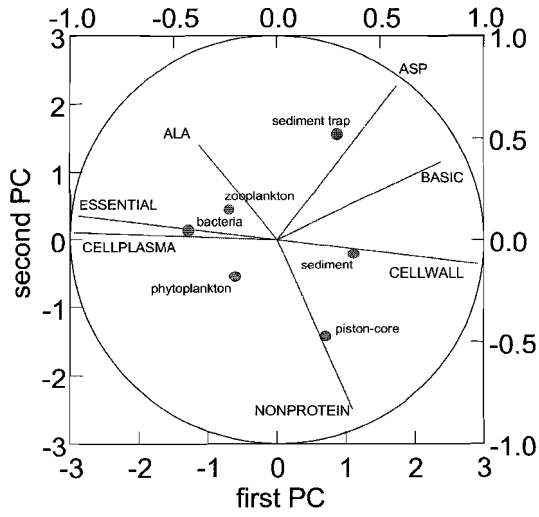

Figure 1.2 Results from a Principal Component Analysis (PCA) applied to normalised data from labile organic matter end-members as bacteria, phytoplankton, zooplankton and to more refractory organic matter from sediment traps, surface sediments and piston cores (representing organic matter of ~18,000 y). Coefficients for standardised factor scores on the 1st and 2nd PC are given for classes of amino acids. The variance along the 1st PC explained 57% of the total variance among the samples, the variance along the 2nd PC explained another 22%.

All of the protein amino acids, except glycine, contain at least one chiral carbon. The chiral carbon contains an amino group (NH_2), a carboxyl group (COOH), a proton (H) and an R-substituent that is different for all amino acids. Amino acids can exist in two configurations that are mirror images (L- and D-amino acids), called enantiomers. Proteins in (almost) all organisms are composed of the L-amino acids that can chemically racemize to its mirror D-enantiomeric configuration. On time scales of 10^5-10^6 years racemic mixtures with equal amounts of L-and D-amino acids will be reached. In aqueous solutions protein-bound amino acids hydrolyse 2-3 times faster than free amino acids. In solid matrices, however, like carbonate shells or bones protein-bound amino acids are racemized slower than free amino acids (Bada and Schroeder, 1975; Collins et al., 1999). The racemization rate is different for each individual amino acid, and depends on pH, temperature and the electron-withdrawing capacity of the R-substituent. In environments where the pH and temperature has been constant for long periods of time, the ratio of D/L amino acids was used to determine ages in a variety of deep-sea samples (Kvenvolden and Etta, 1970; Kvenvolden et al., 1973; Bada and Schroeder, 1975), and in lake sediments (Schroeder and Bada, 1976).

Some D-amino acids (D-alanine, D-glutamic acid, D-aspartic acid and D-serine) are produced by bacteria for peptidoglycans, the main constituents of their cell walls (Meister, 1965; Schleifer and Kandler, 1972). In several studies D-amino acids were detected in particulate and dissolved organic carbon from the water column as well as in sediments that were too young for any significant contribution by racemization (Lee and Bada, 1977; Bada and Lee, 1977; Pollock and Kvenvolden, 1978; Lee, 1992; McCarthy et al., 1998; Pedersen et al., 1999). It was concluded that amino acids from bacterial cell walls may significantly contribute to THAA in the ocean. Deeper in the sediments organic matter may be sequestered as bacterial biomass or as bacterially derived products due to the absence of bacterial grazers (Lee, 1992) and due to the relative resistance of bacterial cell walls against degradation (Rogers, 1983) as was recently demonstrated by Pedersen (1999). The bacterial production of amino acids could have important implications for studying early diagenesis of amino acids, and thus of organic matter, in marine sediments. Part of the amino acids will be derived from *de novo* synthesis by bacteria in the water column and in the sediment, and are not directly derived from primary production in the euphotic layer of the ocean. Bacteria transform degradable organic matter into labile cell plasma compounds and refractory cell walls. This may increase the amount of amino acids that remain after early diagenesis. The ratio of D/L-amino acids may help to identify this bacterial contribution to sedimentary THAA.

A widely applied tool to study organic matter degradation is the fitting of carbon and amino acid concentration profiles in the sediments with diagenetic models describing sedimentation, sediment mixing and first-order degradation (Berner, 1980; Westrich and Berner, 1984). Assuming that the measured concentration profiles are at steady state, the profiles can be described by:

(Eq. 1) $$\frac{dC_L}{dt} = 0 \rightarrow C_L(x) = D_b \frac{\partial^2 C_L}{\partial x^2} - \omega \frac{\partial C_L}{\partial x} - kC_L$$

C_L is the degradable fraction of POC and/or THAA (μg g^{-1}) at depth x in the sediment (cm). In most studies a degradable and an inert POC and/or THAA fraction are taken into account (Henrichs and Farrington, 1987a; Haugen and Lichtentaler, 1991; Cowie et al., 1992c; Mayer and Rice, 1992; Wang et al., 1998). At a certain depth in the sediment (generally several centimeters) the POC and/or THAA concentration asymptotically approaches a constant value (C_∞) (Fig. 1.3). It is assumed that this background concentration represents the non-degradable pool, and that this pool is constant with depth in the sediment. The remaining fraction (the measured concentration at depth x minus C_∞) is defined as the degradable pool. D_b is the sediment mixing coefficient (cm^2 y^{-1}), which can be derived from fitting ^{210}Pb or ^{234}Th profiles. Sediment mixing of labile organic matter is presumably better represented by the higher ^{234}Th-derived mixing coefficients than by the lower ^{210}Pb-derived values (Smith et al., 1993). ^{234}Th has a decay rate (half-live of 24 days) close to the degradation rate of labile organic matter (~10 y^{-1}) and is, therefore, a sensitive indicator for short-term particle input and mixing. ω is the sedimentation rate (cm y^{-1}), which can be obtained from ^{210}Pb. k is the first-order degradation rate (y^{-1}) for the degradable POC and/or THAA fraction.

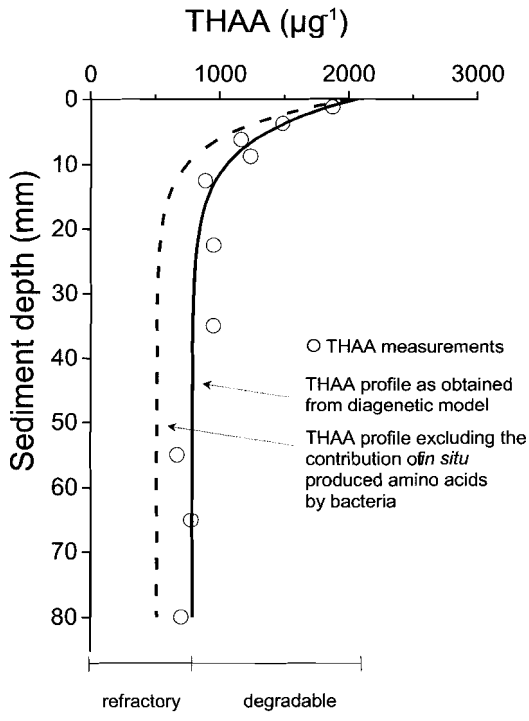

Figure 1.3 Amino acid concentration profile with sediment depth. The solid line through the measured data denotes the result of the diagenetic model (Eq. 1), that fits the THAA concentration in the sediment as a function of sedimentation, sediment mixing and first-order degradation. The shaded area represents the non-degradable pool. The dashed line represent the amount of amino acids in the sediments remaining after lowering for the contribution of amino acids derived from *in situ* produced bacterial amino acids. Thus, the difference between the solid and dashed line is the contribution of bacterial amino acids to THAA. The distribution between the degradable and non-degradable pool would be different when taking into account the contribution of bacterial amino acids.

With the sedimentation rate and sediment mixing coefficient obtained independently from fitting ^{234}Th and/or ^{210}Pb profiles, the first-order degradation constant k remains the only unknown parameter and can be obtained from fitting the measured POC and THAA profiles. *In situ* bacterial synthesis of amino acids, as described above, flattens or steepens (depending on the distribution of bacteria in the sediment) the THAA profile compared to what would have been derived from settling organic matter only (Fig. 1.3). Rather than comparing single k values of POC (or THAA), the ratio of k_{THAA}/k_{POC} is a more reliable estimate of the organic matter diagenetic state since this ratio is insensitive for differences in local conditions between different sites (sedimentation, anoxia, sediment mixing, porosity, etc.). Ratios of k_{THAA}/k_{TOC} were compared in coastal sediments ranging from oxic to suboxic to (seasonally) anoxic, and they all varied between 1 and 1.5 (Henrichs and Farrington, 1987a; Mayer and Rice, 1992; Cowie et al., 1992c). It illustrates that THAA are generally more reactive than bulk POC. In oxic sediments in the Oslofjord, however, the ratio was substantially lower (0.23) than in the coastal sediments (Haugen and Lichtentaler, 1991), which was attributed due to the deposition of substantially degraded material to the sediments in the Fjord and incorporation of amino acids in humic compounds.

Thesis outline

For this thesis samples have been used from two sites at the northeastern Atlantic continental margin (Fig. 1.4). In chapters 2 and 4 results are presented from the Goban Spur continental slope, as part of the OMEX (Ocean Margin Exchange I) programme. The OMEX programme was established within the European Commission's MAST (MArine Science and Technology) Programme. The main goal of OMEX was to address physical, biological, chemical and sedimentological processes along the northeastern Atlantic shelf break. In chapters 3 and 5 results are presented from the southeastern slope of the Faeroe-Shetland Channel (FSC), as part of the PROCS (PROcesses on the Continental Slope) programme. This programme was funded by the Netherlands Organisation for Scientific Research (NWO). The main goal of PROCS was to study the interactions between bottom topography in the FSC, the occurrence and distribution of internal waves and the associated effects on sediment resuspension and cross slope variations of organic matter deposition.

The main research questions addressed in this thesis are:

- Can the distribution of amino acids be used to assess the organic matter degradability across the NE Atlantic continental slope, and is the difference in amino acid distributions related to cross-slope particle size distributions?
- What is the significance of down slope amino acid transport and deposition across the continental slope in addition to vertical settling from the euphotic layer of the ocean?
- Does *in situ* bacterial production of amino acids contribute to the THAA in the sediments and what is the consequence for amino acid degradation rates as obtained from diagenetic modelling?

Chapter 2

Data are presented on TOC, TN and THAA in bulk and size-fractionated sediments across the Goban Spur continental slope. The aim of the study is to establish whether amino acids can be used to assess the organic matter degradability across the slope and to examine whether this degradability is related to cross-slope particle size distributions. An important tool to study the organic matter degradability is the application of a diagenetic 'reaction-diffusion' model. A diagenetic model is applied to sedimentary TOC, THAA and individual amino acid profiles to evaluate the THAA reactivity with respect to that of TOC and to study the contribution of individual amino acid to overall THAA degradation. In addition to the 'reaction-diffusion' model, a Principal Component Analysis (PCA), applied to amino acid mole percentages, was carried out to test whether there is any statistical trend in the distribution of amino acids in bulk sediments and size fractions across the continental slope.

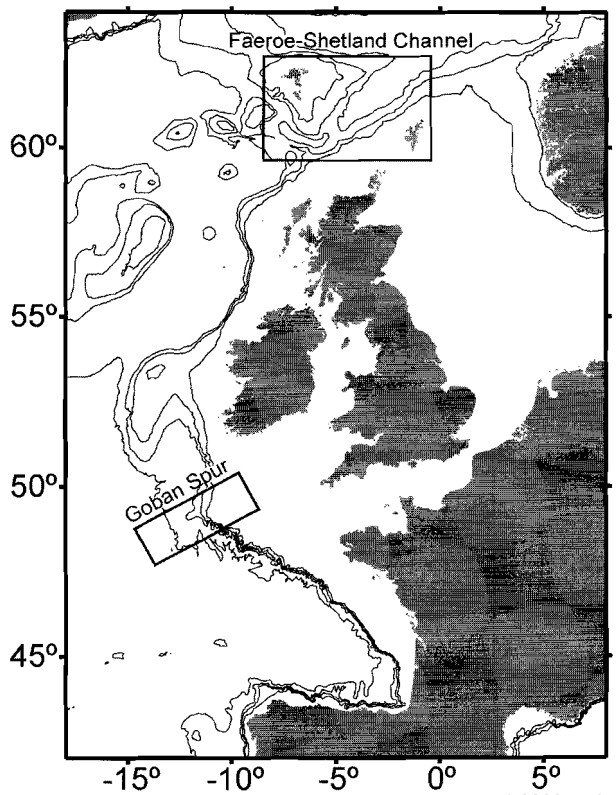

Figure 1.4 The NE Atlantic continental margin with the 200, 500, 1000 and 3000 m isobaths. Samples were collected from the Goban Spur area, along a transect from 49°24.89'N, 11°31.42'W (650 m) to 49°05.30', 13°26.18'W (3650 m) and from the Faeroe-Shetland Channel, along a transect from 60.73°N, 2.87°W (300 m) to 61.00°N, 3.18°W (1000 m).

Chapter 3

Data are presented on TOC, TN and THAA in suspended matter from the water column, sediment traps and surface sediments (bulk and fractionated) from the PROCS 99-1 cruise across the SE slope of the FSC. The aim of the study is to determine the significance of down-slope transport of amino acids in addition to the vertical settling from the euphotic layer of the ocean. An end-member model, based on TN, THAA and amino acid mole-percentages in suspended matter samples from the water column, sediment trap samples and surface sediments, is described. The model is used (1) to study the relative contribution of amino acids from vertical settling to the THAA in the sediment surface during periods of low sediment resuspension and (2) to examine if amino acids from the sediment surface were resuspended during periods of increased bottom currents. A PCA is applied to amino acid distributions in the samples to assess the organic matter degradability across the slope. Data on grain-size analysis, ^{234}Th, TOC, TN and THAA in the sediments are combined to study cross-slope erosion-deposition patterns of amino acids and the associated variability of the organic matter degradability.

Chapter 4

Data are presented on THAA and amino acid enantiomers in organic matter samples from sediment traps, the sediment surface and samples from deeper sediment layers (corresponding to ages of 18 ky) across the Goban Spur continental slope. The aim of the study is to examine the contribution of bacterially synthesised amino acids to THAA. First, the contribution of D-amino acids by racemization is identified by application of a 'sedimentation-mixing-degradation-racemization' model. Then, from the remaining D-amino acid concentration the potential contribution of bacterially derived amino acids, either from whole cells or from cell wall remnants, is estimated by using literature values of D/L ratios from bacterial cultures and pure peptidoglycan. A diagenetic 'diffusion-reaction' model is applied to original THAA concentration profiles and to profiles that are lowered for the bacterially derived amino acids to study whether ignoring the contribution of *in situ* synthesised amino acids results in erroneous estimates of degradation rates.

Chapter 5

Data are presented on THAA and amino acid enantiomers in suspended matter samples from the water column, sediment trap samples and bulk and size-fractionated sediments across the SE slope of the FSC. The aim of this study is to test the hypothesis that part of the mid-slope accumulation of D-amino acids is associated with bacterial cell walls that are produced in near-bottom water layers. An end-member model based on D-amino acids, similar to that presented in Chapter 3, is used to evaluate if the distribution of D-amino acids across the FSC corresponds to that of THAA. The contribution of D-amino acids by racemization in the sediment is estimated by application of a 'sedimentation-mixing-degradation-racemization model. The contribution of bacterially derived amino acid to THAA is estimated from the concentration profiles of D-amino acids, lowered for racemization, by using literature values of D/L-amino acids for whole cells as well as for cell wall peptidoglycan. Data on amino acid enantiomers and mole-percentages of non-protein amino acids are combined to examine whether the contribution of bacterially derived amino acids to THAA increases with increasing degradation of the organic matter.

2.

Total hydrolysable amino acid mineralisation in sediments across the NE Atlantic continental slope (Goban Spur).

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Abstract

Total hydrolysable amino acids (THAA), individual amino acid distributions, total organic carbon (TOC) and total nitrogen (TN) were measured in sediments across the Goban Spur continental slope at water depths of 651 m, 1296 m and 3650 m. Objectives were to examine (1) differences in organic matter (OM) degradation state in surface sediments across the slope from sedimentary amino acid compositions, and (2) whether these differences are related to particle size distributions. Application of a 'reaction-diffusion' model to the sediment concentration profiles showed that TOC and THAA degradation rate constants decreased with increasing water depth. Ratios of THAA degradation over TOC degradation indicated that THAA turn over faster than TOC at 651 and 1296 m water depth only. From estimates of degradation rate constants of individual amino acids, it was concluded that with increasing water depth fewer amino acids contribute to overall THAA degradation. Although the concentration of amino acids increased the THAA mineralisation rate as well as the contribution of THAA to TOC mineralisation decreased with depth across the slope. This illustrates that the overall amino acid reactivity decreases with increasing water column depth. A Principal Component Analyses, carried out on normalised amino acid mole percentages, established significant shifts in amino acid compositions. The PCA confirmed that (1) OM degradation state increased from 651 to 3650 m and (2) that OM in the finest fraction at the shallowest station appeared to be considerably less degraded than in the coarser fractions or any size fractions at the deeper stations. Therefore, we conclude that down slope transport, sorting and accumulation of fine particles with continuous mineralisation of OM attached to the particles during vertical and lateral transport results in an increasing organic matter degradation state from the upper slope to the abyssal plain.

Introduction

Organic matter (OM) is usually transported through the water column in the form of aggregates attached to sinking particles (Lee and Cronin, 1984; Ransom et al., 1998; Honjo, 1997). Newly formed aggregates of OM are rich in labile organic matter, thus providing a suitable substrate for heterotrophic bacteria, and are therefore sites of active mineralisation (Alldredge, 1989; Alldredge and Gotschalk, 1990; Alldredge et al., 1993). As much as 30-40% of the annual water column productivity might reach the sediments in shallow areas of high primary productivity, however; this amount sharply decreases with increasing water depth (Middelburg et al., 1997).

Nitrogen-rich compounds of OM such as amino acids are generally considered to be degraded faster than nitrogen-poor compounds (e.g. lipids). This preferential utilisation

of amino acids causes the contribution of amino acid nitrogen to total nitrogen (TN) to decline from 75-90% in fresh plankton to 40-50% in OM in sinking aggregates to 10-30% in sedimentary OM (Lee, 1988). In addition, several studies of amino acids in sediment trap material (Lee, 1988; Haake et al., 1992; Haake et al., 1993; Nguyen and Harvey, 1997) and sediments (Henrichs et al., 1984; Sugai and Henrichs, 1992; Cowie and Hedges, 1992b; Boski et al., 1998; Dauwe and Middelburg, 1998) have indicated that the relative contribution of individual amino acids to THAA changes during OM mineralisation. These studies established that neutral amino acids (valine, methionine, phenylalanine, isoleucine, leucine) are preferentially mineralised. Non-protein amino acids (β-alanine and γ-aminobutyric acid) are present in marine organisms in small quantities only, and their relative amount increases with ageing of OM as a result of the decarboxylation of their precursor acidic amino acids (Lee and Cronin, 1982). Basic amino acids (lysine, histidine, arginine) adsorb relatively easily onto available mineral surfaces (Hedges and Hare, 1987; Henrichs and Sugai, 1993; Wang and Lee, 1993) and are therefore assumed to be less susceptible to microbial degradation (Schuster et al., 1998). Thus, the amino acid composition is a valuable tool to assess the OM degradation state (Dauwe and Middelburg, 1998).

The amount and composition of THAA ultimately delivered to the sediments is strongly associated with specific surface area and hence with particle size (Mayer, 1994a; Mayer, 1994b; Hedges and Keil, 1995). After arrival at the sediment surface, repetitive cycles of sedimentation-resuspension and disaggregation-aggregation hydrodynamically sort small OM-rich particles from coarser OM-poor particles (Thomsen and Van Weering, 1998; Thomsen, 1999). Therefore, the THAA concentration and relative contributions of individual amino acids in 'bulk' sediments are expected to change together with particle size distributions across the continental slope.

We present results of total organic carbon (TOC), TN and THAA in bulk sediment samples as well as in four sediment fractions (corresponding to approximately <0.5 μm, 0.5-2 μm, 2-10 μm and >10 μm) from 3 stations across the Goban Spur continental slope that were obtained as part of the EC-MAST II Ocean Margin EXchange (OMEX I) project. Within the scope of the OMEX project, we wanted to examine whether there are differences in organic matter degradation state at different stations across the Goban Spur continental slope. Since the concentration as well as the relative contribution of amino acids are known to change during mineralisation and are expected to change with particle size distribution, it was our main objective to establish whether amino acids can be used to further evaluate the OM quality at the Goban Spur continental slope. An important tool for the study of OM quality was the application of a diagenetic 'reaction-diffusion' model. Sediment mixing and first-order degradation rate constants, derived from the model, were used to assess differences in THAA and TOC reactivity as well as to assess the contribution of amino acid to overall OM mineralisation. A Principal Component Analysis with amino acid mole percentages was carried out to test whether there is any statistical trend in the distribution of amino acids in bulk sediments and size fractions with increasing distance from the shelf-break

Materials and methods

Sediment samples were collected at the northeastern Atlantic continental slope (Goban Spur) with R.V. Pelagia in September 1995. The Goban Spur (49°24.89'N, 11°31.42'W – 49°05.30'N, 13°26.18'W) is located at the southwestern edge of the Celtic Sea with a gradually increasing water depth from 200 m to 1300 m on the upper slope, followed by a steeper lower slope down to 4850 m towards the Porcupine Abyssal Plain. The morphology and hydrography of the margin are described in detail by Van Weering et al. (1998).

Sediment sample preparation

At depths of 651 m (PE-95-9), 1296 m (PE-95-6) and 3650 m (PE-95-8) cores were taken with a multicorer (i.d. 62 mm) and processed immediately, at *in-situ* temperature, in a thermostated lab (Lohse et al., 1998). The upper 10 mm of the sediment cores were sliced into slices of 2.5 mm, sediment from 10-30 mm into slices of 5 mm, from 30-60 mm into slices of 10 mm, and from 60-140 mm into slices of 20 mm. Further description of sediment sample preparation has been given by Lohse et al. (1998).

Total organic carbon (TOC) and total nitrogen (TN)

Splits of frozen sediment slices were thawed, oven-dried at 60°C, thoroughly homogenised and analysed on a Carlo-Erba 1500 elemental analyser following the procedure of Verardo et al. (1990) after removing carbonates by addition of sulphurous acid. Further description of the TOC and (non-acidified) TN analysis has been given in Lohse et al. (1998; 2000).

Sediment fractionation

Bulk sediment was separated by hydrodynamic properties (size, shape and density) of sediment particles, after Keil et al. (1994a), with an ultracentrifuge into four classes corresponding to approximately <0.5 μm, 0.5-2 μm, 2-10 μm and >10 μm. Splits of ~10 g of frozen sediment were thawed and suspended in 100 ml NANOpure (>17 m Ω) water in acid cleaned glass centrifuge tubes. The suspension was centrifuged for 14.4 minutes at 1500 rpm, which separated the finest particles from larger particles. The suspension containing the finest fraction was siphoned into an acid cleaned 1-litre Erlenmeyer. This step was repeated 10 times. The sediment residue was suspended again and centrifuged at 1000 rpm for 1.8 min and at 200 rpm for 1.8 min to separate the ~0.5-2 μm and ~2-10μm fractions. The remaining particles formed the coarse fraction. Approximately 50 ml of a 0.1 M $CaCl_2$ solution were added to the collected size fractions to stimulate sedimentation. Samples from the overlying water were analysed for THAA and indicated that only ~1% of the THAA were released from the sediment during size fractionation. After removal of the overlying water, the sediment was 'washed' with NANOpure water to remove the Ca^{2+} ions, suspended again in 10 ml NANOpure water and kept frozen until TOC, TN and THAA analysis. Suspensions of the sediment size fraction were also analysed by XRD for mineralogical composition. A mass balance, obtained by repeating the fractionation procedure with a suspension of oven-dried sediment (60°C), showed that the recovery was better than 98.5%.

Amino acids

Bulk and size fractionated sediment samples were measured for amino acids by reverse-phase HPLC analysis of their fluorescent derivatives as described by Lindroth and Mopper (1979) and Cowie and Hedges (1992a) and modified after Dauwe and Middelburg (1998). Briefly, 5 ml of 6 M HCl was added to ~0.25 g dried sediment in 8 ml autoclavable glass screw cap vials. The suspension was flushed with N_2 for at least three minutes. Hydrolysis took place at 110°C for 24 h. Hydrolysates were filtered through 0.45 µm Gelman syringe filters (low protein binding). The filtrate was diluted in acetic acid buffer (pH 6.8) and frozen at -20°C until analysis. Blanks, containing 5 ml 6 M HCl, were treated the same way as sediment samples.

Prior to HPLC analysis, pre-column reaction of amino acids with *o*-Phtaldialdehyde (OPA), buffered to a pH of 9.5, was used to form fluorescent derivatives of the amino acids (Lindroth and Mopper, 1979). The HPLC system consisted of a Merck-Hitachi pump (L-6200A), a Merck-Hitachi autosampler (AS-4000) and a Merck-Hitachi fluorescence detector (F1050). A reverse-phase Hypersil ODS 2 C_{18} column with Nova-Pak C_{18} Guard-Pak inserts and a binary solvent system were used to separate seventeen amino acids during a run of 40 minutes. The binary solvent system consisted of HPLC-grade methanol (A) and acetic acid buffer (pH 6.8) with 2% THF and 10% methanol (B). With a flow rate of 1 ml min^{-1}, the amount of solvent A was increased from 0 to 25% in 15 minutes, then to 60% in 11 minutes. After 2 minutes, the amount of solvent A was increased from 60% to 100% in 1 minute, and decreased to 0% again after 5 minutes. Before the next sample was analysed, the HPLC system was flushed with 100% of solvent B for four minutes to decrease the pressure in the system and to stabilise the base line. The retention times of glycine and threonine drifted slightly during the analysis as a consequence of temperature changes and were, therefore, treated as one component when they eluted together.

Amino acids were fluorimetrically detected at an excitation wavelength of 328 nm and an emission wavelength of 450 nm. Concentrations of the amino acids were calculated from peak areas, which were calibrated with a standard amino acid mixture (AA-S-18, SIGMA chemicals) ranging in concentration from 150 to 1500 nM. Two amino acids, β–alanine (PAA-11, SIGMA chemicals) and γ-aminobutyric acid (PAA-9, SIGMA chemicals), were added as standards for the non-protein amino acids. No internal standards were used to account for losses that may occur during hydrolysis or analysis. The average THAA concentration of the sample blanks never exceeded 1% of the lowest THAA concentration in the samples. All samples were analysed in duplicate. For all amino acids, the correlation between peak areas and standard concentration was good (r^2>0.95), except for lysine, which had a slightly lower correlation coefficient (r^2=0.88). The analytical precision was 5-10% for the acidic and most neutral amino acids; and 10-15% for valine, iso-leucine, the basic lysine and histidine and the non-protein γ-aminobutyric acid. The precision for tyrosine was ±33%.

Degradation rate constants and mineralisation rates

A simple model including sediment mixing and first-order degradation was used to fit the TOC, THAA and individual amino acid concentration profiles. Sediment accumulation was low (2.8, 1.7 and 4.3 cm ky^{-1} at 651, 1296 and 3650 m; Van Weering et al., 1998) and

advective transport was therefore neglected (Lohse et al., 1998; Soetaert et al., 1996). Assuming that OM concentration profiles were at steady state they were described by

(Eq. 1) $$\frac{\partial C_L}{\partial t} = 0 = D_b \frac{\partial^2 C_L}{\partial z^2}$$

where C_L is the concentration of labile OM (TOC, THAA or individual amino acids) per volume of bulk sediment (g cm^{-3}). This is defined as the total concentration of TOC, THAA or individual amino acids minus the concentration of the non-degradable part of the same component (which is assumed to be constant with depth) (C_{TOT} - C_{ND}). D_b is the sediment mixing coefficient (cm^2 y^{-1}); z is the depth in the sediment, positive downwards (cm); and k_L is the first-order degradation rate constant (y^{-1}) of the labile OM pool. Porosity changes with sediment depth are moderate at the Goban Spur (Lohse et al., 1998)) and were neglected for simplicity. Non-local mixing (Soetaert et al., 1996) was not taken into account. Under the boundary conditions that $\partial C_L/\partial z \rightarrow 0$ (or $C_{TOT} \rightarrow C_{ND}$) as $z \rightarrow \infty$ and $C_{TOT} = C_{TOT,0}$ (and $C_L = C_{L,0}$) at z = 0, the solution of equation (1) is

(Eq. 2) $$C_L(z) = C_{L,z_0} e^{-(\sqrt{\frac{k}{D_b}})z} + C_{ND}$$

By assuming that the bulk OM consists of a degradable pool with k_L and a non-degradable pool (k_{ND}=0), a bulk OM degradation rate constant can be derived from the equation $k_{bulk}C_{TOT} = k_L C_L$, which at z = 0 yields $k_{bulk} = (kC_{L,0})/C_{TOT,0}$.

To determine the model parameters C_{TOT}, C_{ND} and $\sqrt{(k/D_b)}$, equation 2 was fitted through the measured TOC concentration profiles by using the Excel solver routine (least squares). To estimate D_b and k independently we assumed that TOC mineralisation rates (J_o) equalled the oxygen flux, after conversion from oxygen to carbon units (C:O is 106:138), as determined from oxygen microprofiles by Lohse et al. (1998), since oxic degradation accounted for 68-90% of the TOC mineralisation rates on the upper slope of the Goban spur and for more than 90% at the lower slope. The equation for TOC mineralisation can be derived from equation 2 according to Fick's first law:

(Eq. 3) $$J = -D_b \frac{\partial [C_L(z)]}{\partial z}$$

which at z = 0 yields:

(Eq. 4) $$J_0 = C_L \sqrt{kD_b}$$

If the TOC mineralisation rates, as derived from the flux of oxygen, are applied to Eq. 4, we have two equations with two unknowns, and hence D_b and k can be calculated. The sediment mixing coefficients (D_b) derived from the TOC concentration profiles were used to fit THAA concentration profiles to ultimately calculate the THAA degradation constants. The flux of THAA and individual amino acids were then calculated from Eq. 4.

Results

Organic carbon and total nitrogen concentrations

The TOC concentration in the top layer (0-2.5 mm) was 3100 μg g^{-1} at the upper slope (651 m), 6400 μg g^{-1} at the continental break (1296 m) and 4400 μg g^{-1} at the lower slope (3650

m). The concentration profiles (Fig. 2.1) showed most pronounced down-core gradients at 1296 m, reaching background values at about 30 mm. Patterns of TN were similar to those of TOC.

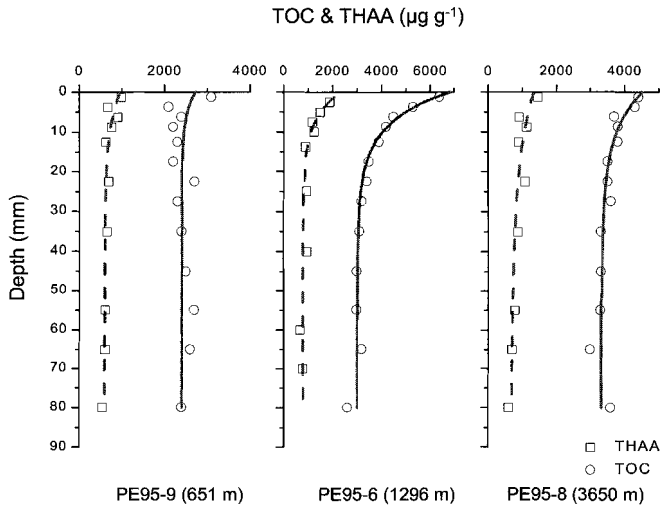

Figure 2.1 TOC (○) and THAA (□) concentration profiles at PE95-9 (651m) PE95-6 (1296m) and PE95-8 (3650m). Solid and dotted lines: concentration profiles fitted through measured TOC and THAA, with a diagenetic model describing sediment mixing and first-order degradation. Note the differences in scale between the sampling stations.

Total hydrolysable amino acid concentrations

THAA concentration profiles followed the same trend as TOC (Fig. 2.1). The THAA concentration in the top layer at 651 m was 982 µg g^{-1} and decreased gradually to 534 µg g^{-1} at 80 mm sediment depth. The highest THAA concentration was measured at 1296 m where THAA decreased from 1876 µg g^{-1} at the surface to 704 µg g^{-1} down core. At 3650 m the THAA concentration decreased from 1451 µg g^{-1} in the upper layer to 600 µg g^{-1} down core.

Relative contribution of amino acids to organic carbon and total nitrogen

At 651 m, the contribution of THAA-carbon to TOC (%-AAC) decreased from ~13% in the top layers to ~10% at the 70-90 mm interval (Fig. 2.2), with a subsurface peak at ~7 mm. At 1296 m values scattered around 10-12% without a trend with sediment depth. At 3650 m, the %-AAC varied between 10 and 15% in the top layers and was ~7% at 70-90 mm. Patterns of THAA-nitrogen to TN (%-AAN) closely followed the distribution patterns of %-AAC. Values scattered around 30% at the stations at 651 m and 1296 m with a subsurface peak of ~45% at 651 m. At 3650 m there was more scatter in the %-AAN, and it ranged between 20 and 38%.

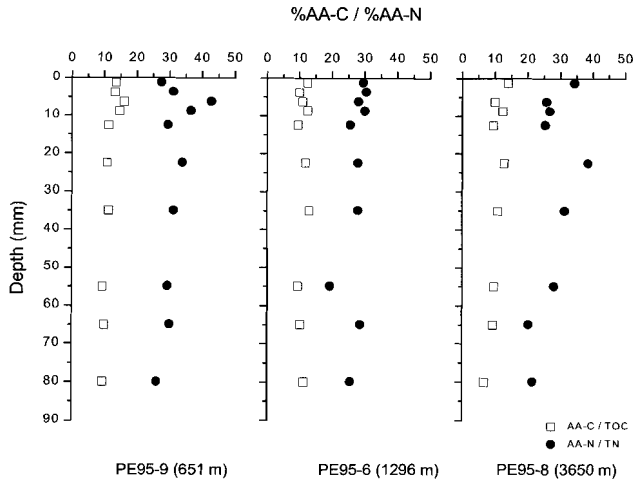

Figure 2.2 Contribution of amino acid-carbon to TOC (%-AAC; □) and amino acid–nitrogen to TN (%-AAN; ●) at 651 m, 1296 m and 3650 m.

Amino acid mole percentages in bulk sediment

Fig. 2.3 shows mole percentages of the seventeen individual components at the three stations as a function of sediment depth. At all three stations G+T (the sum of glycine and threonine) is the most abundant compound (25-30%), followed by aspartic acid, alanine, glutamic acid and serine. Together these compounds contributed more than 70% to THAA. At all three stations, there were no significant trends in relative contributions of individual amino acids with sediment depth.

Ratios of aspartic acid to its derivative β-alanine scattered around 10 at the shallowest station without a significant trend with sediment depth (Fig. 2.4). At the deeper stations, lower ratios of about 5 were calculated, except for one elevated ratio in the sediment top layer at 3650 m. The ratio of glutamic acid to its derivative γ-aminobutyric acid (Fig. 2.4) scattered around 8-15 at all sediment depths at each station, with the exception of a value of ~23 at the 60-70 mm interval and a value ~45 at the 30-40 mm interval at the shallowest station.

Although the differences were relatively small, some distinct patterns appeared in the sediment top layers of the three stations (Fig. 2.5). G+T, alanine and β-alanine mole percentages were almost similar at 651 m and 3650 m, whereas the contributions were elevated at 1296 m. Aspartic acid, and to a lesser degree valine, phenylalanine, isoleucine, leucine and lysine mole percentages were lower at 1296 m relative to the other stations. Mole percentages of the other amino acids were nearly constant at all stations.

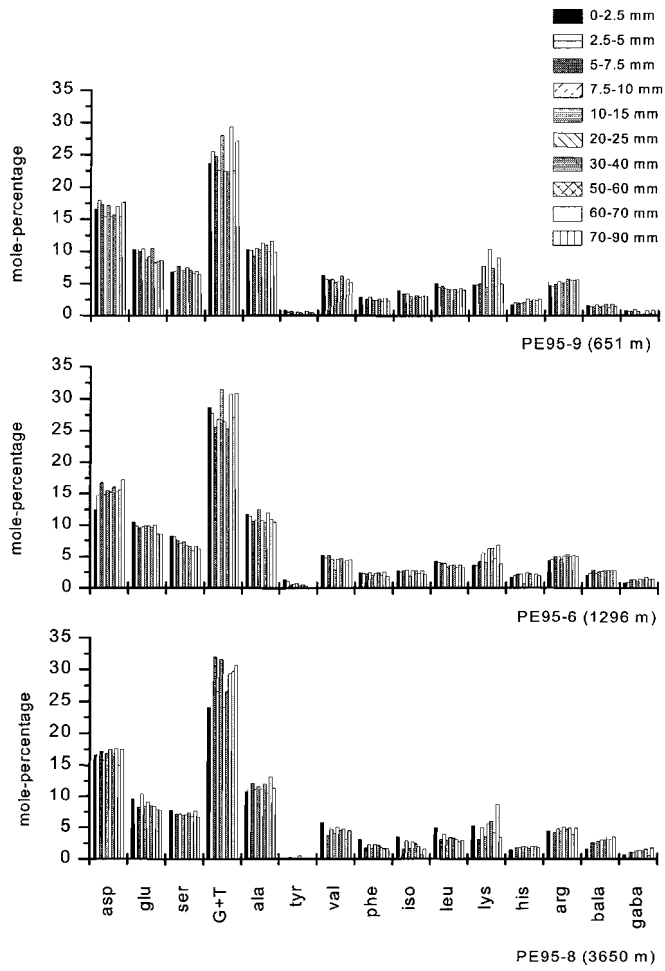

Figure 2.3 Amino acid mole% in sediments at 651 m, 1296 m, and 3650 m. Acidic amino acids: aspartic acid and glutamic acid. Neutral amino acids: serine, glycine + threonine (G+T), alanine, tyrosine, valine, phenylalanine, isoleucine and leucine. Basic amino acids: lysine, histidine and arginine. Non-protein amino acids: β-alanine and γ-aminobutyric acid.

Model derived mixing coefficients, degradation rate constants and mineralisation rates

Model results of TOC and THAA are given as solid lines in Fig. 2.1. Sediment mixing coefficients, derived from TOC concentration profiles, were 2.6 $cm^2\ y^{-1}$ at 651 m, 0.4 $cm^2\ y^{-1}$ at 1296 m and 1.3 $cm^2\ y^{-1}$ at 3650 m (Table 2.1). Calculated degradation rate constants of labile TOC (k_L) varied between 0.7 and 8.4 y^{-1} with highest values at 651 m. Estimated values of k_{bulk} spanned a smaller range (0.3-1.0 y^{-1}) with again the highest value upslope

(Table 2.1). Degradation rate constants for THAA showed the same trend (k_L = 0.4-3.2 y^{-1}, k_{bulk} = 0.2 – 1.2 y^{-1}; Table 2.1).

First-order degradation constants for individual amino acids decreased with increasing water depth (Fig. 2.6, upper panel). At 651 m, degradation rate constants of all amino acids (except serine, lysine and histidine) were well above 1 y^{-1}, whereas at 3650 m only degradation rate constants of some neutral acids reached this value, suggesting that fewer amino acids participated in THAA degradation with increasing water depth. The goodness of fit of the model (expressed as r^2) was 0.7-0.85 for most of the individual amino acids (Fig. 2.6, lower panel). Exceptions were tyrosine, lysine, histidine, arginine and γ-aminobutyric acid at 651 m, lysine and histidine at 1296 m, and lysine, β-alanine and γ-aminobutyric acid at 3650 m.

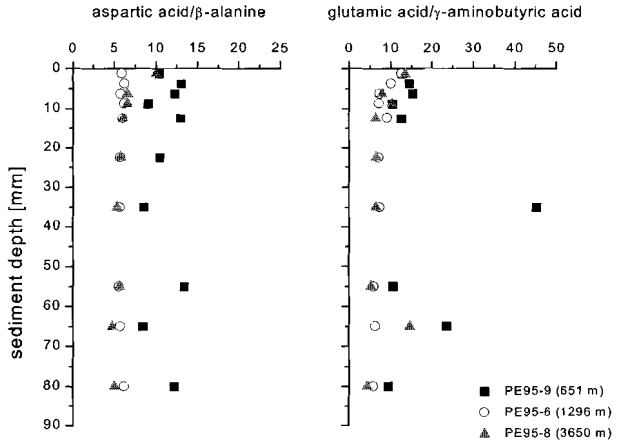

Figure 2.4 Ratios of aspartic acid and glutamic acid to their non-protein derivatives β-alanine and γ-aminobutyric acid at the three stations across the slope (651 m, 1296 m and 3650 m).

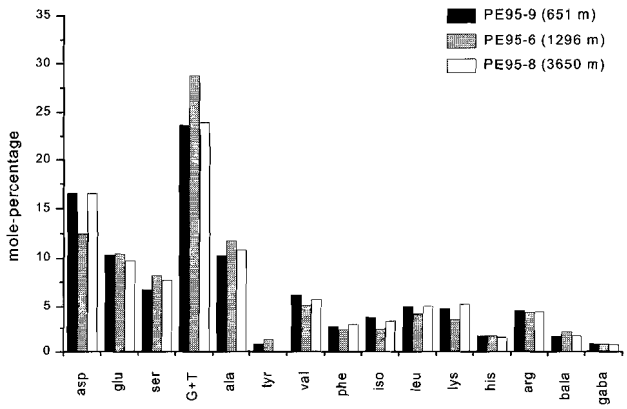

Figure 2.5 Amino acid mole% in the sediment top layer (0-2.5 mm) at the three stations across the slope (651 m, 1296 m and 3650 m).

Mineralisation rates (J_0) of THAA were 0.74 mmol m^{-2} d^{-1} at 651 m, 0.31 mmol m^{-2} d^{-1} at 1296 m and 0.22 mmol m^{-2} d^{-1} at 3650 m. The contribution of the THAA mineralisation to the TOC mineralisation decreased from 31% at 651 m to 14% at 3650 m. Maximum mineralisation rates of individual amino acids decreased from 0.23 mmol m^{-2} d^{-1} for aspartic acid at 651 m to 0.078 mmol m^{-2} d^{-1} for G+T at 1296 m, and to 0.064 mmol m^{-2} d^{-1} for G+T at 3650 m. Four amino acids accounted for ~60% of the THAA mineralisation in the decreasing order of aspartic acid > G+T > valine > glutamic acid at 651 m, G+T > leucine > serine > phenylalanine at 1296 m, and G+T>glutamic acid>alanine>serine at 3650 m.

Table 2.1 Sediment characteristics of PE95-9 (651 m), PE95-6 (1296 m) and PE95-8 (3650 m).

		PE95-09	**PE95-06**	**PE95-08**
Latitude		49°25.0	49°11.4	49°05.2
Longitude		11°31.3	12°44.4	13°26.2
water depth	[m]	651	1296	3650
sedimentation rate [(a)]	[cm ky^{-1}]	2.8	1.7	4.3
porosity [(a)]	[-]	0.74	0.85	0.83
median grain size [(a)]	[mm]	123.1	10.9	7.3
TOC (top layer)	[mg g^{-1}]	3.1	6.4	4.4
TN (top layer)	[mg g^{-1}]	0.5	0.9	0.6
THAA (top layer)	[mg g^{-1}]	0.98	1.88	1.45
TOC mineralisation rate[(b)]	[mmol m^{-2} d^{-1}]	2.36	1.78	1.55
THAA mineralisation rate	[mmol m^{-2} d^{-1}]	0.74	0.31	0.22
Sediment mixing	[cm^2 y^{-1}]	2.60	0.39	1.31
k-TOC (labile)	[y^{-1}]	8.42	0.67	1.22
k-TOC (bulk)	[y^{-1}]	0.99	0.38	0.32
k-THAA (labile)	[y^{-1}]	3.24	0.95	0.4
k-THAA (bulk)	[y^{-1}]	1.23	0.59	0.20

(a) Van Weering et al. (1998)
(b) Lohse et al. (1998)

Amino acids in sediment size fractions

At the three stations, the top layers (0-2.5 mm) and the 70-90 mm interval were separated into four classes corresponding to approximately <0.5, 0.5-2, 2-10 and >10 μm, and the fractions were analysed for TOC, TN and THAA. The contribution of the coarse fraction (>10 μm) decreased from 96% in the sediment surface at 651 m to 79% at 3650 m, and at all three stations the contribution of the coarse fraction decreased from the sediment surface to the 70-90 mm interval (Table 2.2). The contribution of the finest fraction (<0.5 μm) in the sediment top layer increased from <0.5% at 651 m to 1% at 3650 m. THAA concentrations were highest in the finest fraction in the sediment top layer (0-0.5 mm) and in at the 70-90 mm interval and decreased with increasing particle size. The large difference in THAA concentrations between size classes (11.1 mg g^{-1} vs. 0.8 mg g^{-1} in the finest and coarse fraction, respectively) was most pronounced at 651 m (Table 2.2). For the four size

classes, the THAA concentrations in the top layer (0-0.5 mm) were higher than at 70-90 mm, but this difference was most pronounced for the finest fraction.

%-AAC and %-AAN were calculated in the sediment size fractions of PE95-9 at 651 m (Table 2.2). In the sediment top layer %-AAC was 16% in the finest fraction and distinctly higher than in the other fractions. At the 70-90 mm interval, there was no real trend in %-AAC with size fraction. Patterns in %-AAN were similar to %-AAC.

Clear trends in relative amino acid distributions over the four sediment size classes could be detected only at station 651 m. Here, mole% of glycine and the non-protein amino acids were lowest in the finest fraction (Fig. 2.7), but the neutral amino acids threonine, valine, phenylalanine, isoleucine and leucine were highest in the finest fraction.

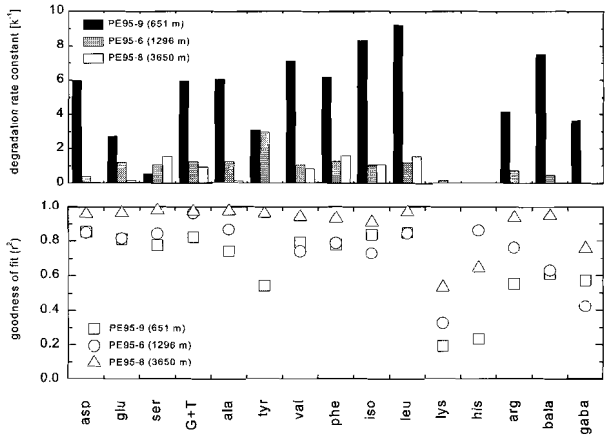

Figure 2.6 Individual amino acid degradation constants from sediments at the three stations across the slope (651 m, 1296 m and 3650 m) are shown in the upper panel. The goodness of fit (r^2) for estimated first-order degradation rate constants of the individual amino acids in sediments from these stations are given in the lower panel. Abbreviations were explained in Fig. 2.3

Discussion

Organic matter is delivered to the sediments by vertical transport through the water column and by lateral transport in the benthic boundary layer. Sinking of particles through the water column is accelerated by aggregation and can be on the order of 100-200 m d^{-1} (Honjo, 1997). Upon arrival at the sediment, aggregates undergo several cycles of resuspension, disaggregation, aggregation and transport before they ultimately become buried in the sediments (Ransom et al., 1998; Thomsen, 1999). During disaggregation-aggregation, OM is continuously mineralised and becomes highly altered (Alldredge, 1989; Alldredge and Gotschalk, 1990; Alldredge et al., 1993). Therefore, it can be hypothesized that the refractory nature of the OM will increase down slope.

For the Goban Spur transect Lohse et al. (1998) found that that TOC mineralisation rates decreased with increasing water depth, in accordance with rates predicted by ocean-wide empirical relationships (Müller and Suess, 1977; Middelburg et al.,

1997). Lohse et al. (1998) and Soetaert et al. (1998) also reported that degradation rate constants of labile and bulk TOC decreased with water depth. Estimates of first-order TOC and THAA degradation rate constants presented in this study show the same trend, confirming the suggested increase in OM degradation state with increasing water depth.

Table 2.2 Particle size distribution in the sediment top layer (0-2.5 mm) and at the 70-90 mm interval. THAA were analyzed in the size fractions (mg g^{-1}) at these intervals. TOC and TN were analyzed in size fractions of PE95-9 (651 m), therefore %-AAC and %-AAN were calculated for this station only.

		top layer (0-2.5mm)				70-90mm			
		< 0.5µm	0.5-2µm	2-10µm	>10µm	< 0.5µm	0.5-2µm	2-10µm	>10µm
PE95-9	fraction (%)	0.23	1.2	2.4	96.1	1.0	1.0	13.0	85.0
PE95-6		1.0	10.0	10.0	79.0	13.3	32.5	12.2	42.0
PE95-8		1.0	10.0	10.0	79.0	2.3	21.7	60.6	15.4
PE95-9	THAA (mg g^{-1})	11.13	6.59	4.90	0.81	2.37	2.10	1.76	0.33
PE95-6		6.95	2.89	4.60	1.51	1.62	0.95	0.47	0.33
PE95-8		4.04	1.57	3.04	1.20	1.82	1.20	0.36	0.50
PE95-9	%-AAC	16.2	11.9	10.5	11.4	9.0	7.5	7.8	11.1
	%-AAN	45.0	30.1	30.1	38.8	20.2	21.5	24.4	24.2

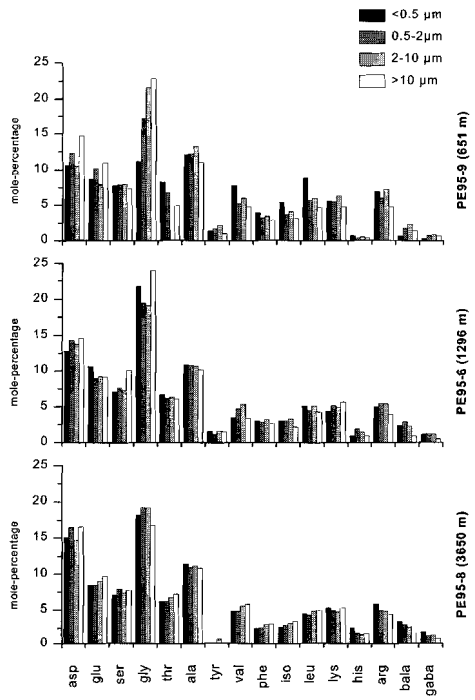

Figure 2.7 Amino acid mole% in the <0.5 µm, 0.5-2 µm, 2-10 µm and >10 µm size fractions of top layer sediments (0-2.5 mm) from stations at 651 m, 1296 m and 3650 m water depth. Amino acids are shown as acidic, neutral, basic and non-protein amino acids from left to right.

Since it is uncertain whether mixing of fresh organic matter is better represented by ^{234}Th or by ^{210}Pb derived sediment mixing coefficients (D_b) (Smith et al., 1993), we decided to independently estimate D_b, and hence the degradation rate constant (k), from the sedimentary oxygen and carbon concentration profiles under the assumption that carbon mineralisation rates equal oxygen consumption rates. TOC degradation rate constants presented in this study are higher than rate constants estimated in sediments from the same area as reported by Lohse et al. (1998), whereas they are lower but more in line with results of Soetaert et al. (1998) and Herman et al. (in press). Although these studies are partly based on the same dataset, the differences between degradation rate constants can be explained considering the boundary conditions that were used in the models. Lohse et al. (1998) calculated TOC degradation rate constants based on ^{210}Pb derived D_b mixing coefficients. Their values are approximately one order of magnitude lower than values presented in this study and hence resulted in lower k values. Soetaert et al. (1998) and Herman et al. (in press) assumed that sedimentary OM could be separated into three fractions: a non-degradable fraction and a degradable fraction that could be further subdivided into a rapidly and slowly mineralisable part. They estimated that the degradable fraction of OM was dominated by the rapidly mineralisable part. Therefore, the k value of this part determined the overall k_{TOC} value, and explained the high degradation rate constants reported in their study. Further, they obtained carbon mineralisation rates from fitting oxygen, nitrate and ammonium profiles. If we apply these total mineralisation rates to our diagenetic model, calculated D_b values would be 1.73-4.4 cm^2 y^{-1}, 0.22-0.39 cm^2 y^{-1} and 0.66-0.89 cm^2 y^{-1} at 651 m, 1296 m and 3650 m water depth, respectively. These values are close to the values given in Table 2.1 and thus indicate that differences in degradation rate constants between the mentioned studies and k values presented here originate mainly from the assumed high contribution of rapidly mineralisable OM. Moreover, since oxic degradation was responsible for 68 to >90% of TOC mineralisation we consider our mixing coefficients (and degradation rate constants) to be a reliable estimate and consequently applied these values to fit THAA concentration profiles.

Sedimentary THAA concentration profiles (Fig. 2.1) followed the trends of the TOC concentration profiles. Boski et al. (1998), who focused on the relationship between amino acids and clay-minerals in the sediments across the Goban Spur, found similar trends in THAA concentrations. Together with the relatively constant values of %-AAN (Fig. 2.5), this indicates that there is no preferential degradation of THAA relative to bulk TOC and that OM was indeed already substantially degraded prior to incorporation into the sediment (Cowie and Hedges, 1992b). THAA degradation rate constants were high at the shallow station and decreased towards the deeper stations, indicating the lower reactivity of the OM delivered to these stations. Burdige and Martens (1988) derived THAA degradation constants from a diagenetic model including sedimentation and neglecting sediment mixing in OM-rich coastal sediments; Mayer and Rice (1992) also calculated protein degradation constants, for a coastal sediment. The degradation rate constants in both studies (1.36 y^{-1} and 1.7 y^{-1}, respectively) are consistent with our k-values for THAA (3.24-0.4 y^{-1}). Wang et al. (1998) fitted TOC and THAA concentration profiles in deep-sea sediments (4100 m) starting at a depth of 5 cm below the sediment mixing zone. Their low k-values of 0.0005 y^{-1} for TOC and 0.0014 y^{-1} for THAA, relative to values presented here, can be explained by the low degradability of aged OM below the mixing zone. Since it is obvious that THAA

degradation constants depend on the model boundary conditions used to fit concentration profiles and on the time window of observation (only top layer vs. sediment depths below the mixing zone), a more reliable parameter to compare the OM degradation at different sites is the k_{THAA}/k_{TOC} ratio. Ratios of k_{THAA}/k_{TOC} in coastal sediments, ranging from oxic to suboxic to (seasonally) anoxic (Mayer and Rice, 1992; Henrichs and Farrington, 1987; Cowie et al., 1992c) all varied between 1 and 1.5. Ratios of k_{THAA}/k_{TOC} in anoxic surface sediments from the Oslofjord were close to 1, whereas in oxic sediments the ratio was 0.23 (Haugen and Lichtentaler, 1991), which is substantially lower than ratios calculated for other coastal sediments. It was suggested that this was due to delivery of degraded material to the sediments in the fjord or incorporation of amino acids in humic compounds. In our study, ratios of k_{THAA}/k_{TOC} were 1.24, 1.57 and 0.61 at 651, 1296 and 3650 m, indicating that THAA turn over faster than TOC at 651 and 1296 m only. This was confirmed by the degradation rate constants of individual amino acids. The highest degradation constants were calculated for the relatively labile amino acids leucine, isoleucine, phenylalanine and valine. G+T, which are part of (rapidly sinking) diatom cell wall material, had relatively high degradation rate constants. Aspartic acid, converted to β-alanine, amongst others, was also rapidly degraded. At 1296 and 3650 m, concentration gradients for most of the amino acids were weak, suggesting low degradation rates relative to mixing. At the deepest station, degradation rate constants could be derived only from concentration profiles of some neutral amino acids, G+T and serine. Therefore, we conclude that with increasing distance from the shelf break fewer amino acids contribute to overall THAA degradation.

The contribution of THAA to TOC mineralisation decreased from 31% at the upper slope to 14% at the lower slope. The acidic amino acids, the neutral amino acids, and G+T accounted for approximately 65-70% of the THAA mineralisation at all stations. Since these amino acids were the major contributors to THAA and because their relative contributions to THAA were higher in sediments with reduced THAA mineralisation rates, we conclude that their reactivity decreases from 651 m to 3650 m water depth.

Despite the decreasing degradation rates, which suggest an increasing OM degradation state across the slope, shifts in mole percentages of the amino acid pool with depth in the sediment and between stations were small. As in other studies, G+T, usually associated with diatoms, were most abundant due to their large contribution to primary production, their relatively high sinking rates and their relative stability (Cowie et al., 1992c; Cowie and Hedges, 1992b; Burdige and Martens, 1988). The contribution of G+T in the top layer of 1296 m was higher than at the other two stations. The contribution of relatively labile amino acids, like the cell plasma compounds glutamic acid, tyrosine and phenylalanine (Hecky et al., 1973), as well as aspartic acid and the neutral amino acids valine, isoleucine and leucine, has often been found to decrease during early diagenesis (Cowie et al., 1992c; Cowie and Hedges, 1992b; Dauwe and Middelburg, 1998). However, they hardly varied with sediment depth at our stations, though their presence in the top layer sediment at 1296 m was lower than at 651 and 3650 m. These lower relative contributions, together with a higher contribution of G+T, would suggest a higher OM degradation state at 1296 m. The recently reported relation between mole% of tyrosine with water depth and redox-related differences in sedimentary OM (Suthhof et al., 2000) was not found here. Our tyrosine mole% were as low as in samples from their bioturbated sediments at the lower slope of the

Pakistan continental margin and rather confirmed the relatively refractory nature of OM at the Goban Spur.

The presence of the non-protein amino acids is often used as an additional indicator for OM diagenesis. An increase of the non-protein amino acids with depth in the sediment was measured at 1296 and 3650 m. Ratios of aspartic acid/β-alanine and glutamic acid/γ-aminobutyric acid were highest at the shallowest station, confirming that OM at 651 m was less degraded than at 1296 and 3650 m. Müller and Suess (1977) reported that adsorption onto mineral particles, rather than preferential mineralisation, determines amino acid compositions in sediments, but little evidence for this was found across the Goban Spur. Although sediments are rich in calcium carbonate, ranging from approximately 40% at 651 m to almost 70% at 3650 m, preferential adsorption of acidic amino acids on calcium carbonate (Müller and Suess, 1977; Carter and Mitterer, 1978) does not seem to be important at the Goban Spur, since there was only a small down core increase in mole% of aspartic acid at 1296 m. An increase of the positively charged lysine, and to a lesser degree arginine, with sediment depth at our three stations, however, could indicate preferential adsorption onto clay mineral particles (Hedges and Hare, 1987; Wang and Lee, 1993). Nevertheless, with respect to the small variations down core and between the three stations, amino acid mole% in bulk sediments appeared inappropriate to confirm the suggested increasing OM degradation state with sediment depth.

Differences in OM quality become clear, however, when THAA concentrations and mole percentages in the sediment size fractions are taken into account. THAA concentrations in the finest fraction were distinctly higher than in the coarser fractions, and this was most pronounced at 651 m. The %-AAN of 45% in this fraction is well above the value considered to be indicative of diagenetic alteration of OM (38%; Cowie and Hedges, (1992b)). Together with the high mole% of the labile amino acids and the low mole% of G+T and the non-protein amino acids, it shows that OM in the finest fraction at 651 m is much less degraded. Although THAA concentrations in the finest fraction at the deeper stations are still considerably higher than in the coarser fractions, mole% of the individual amino acids suggest a relatively refractory nature of the OM at the deeper stations, in contrast to results from 651m.

As did Dauwe and Middelburg (1998) and Dauwe et al. (1999), we applied a PCA to bulk sediment concentrations based on the entire amino acid spectrum to evaluate the OM degradation state. Normalised values for each individual amino acid were obtained by subtracting the average amino acid mole% of all samples from each individual mole% and dividing by their standard deviation. Coefficients for standardised factor scores of the individual amino acids were multiplied with their normalised mole% to calculate a so-called site-score for each sampling station. Normalised data from the 0-80 mm layers, together with data from seven North Sea and Skagerrak stations ranging from relatively fresh to refractory (Dauwe and Middelburg, 1998), and the Porcupine Abyssal Plain (4850 m; Horsfall and Wolff, 1997) were combined to evaluate by PCA the OM degradation state in sediments across the continental slope. Coefficients for standardised factor scores on the first Principal Component (PC) explained 48% of the total variance in the data; the second PC explained another 27%. To be consistent with Dauwe and Middelburg (1998), we excluded lysine from our data. Sediment site scores on the first PC (Table 2.3) of the North

Sea stations decreased from +1.16 for a coastal station to –0.19 for the Skagerrak. Site scores of the Goban Spur sediments decreased from –0.71 at 651 m to –1.31 at 3650 m and finally to –1.49 for the Porcupine Abyssal Plain. There was good correlation between mole% of the labile amino acids (tyrosine, valine, phenylalanine, isoleucine and leucine) and site-scores on the first PC (Table 2.3, Fig. 2.8 and 2.9A), demonstrating the decreasing contribution of these amino acids with increasing OM degradation state. Results of the PCA showed that although the differences between the three Goban Spur stations were rather small, OM degradation state increased from 651 to 3650 m. The OM at all three stations appeared more refractory than at any of the shallow North Sea and Skagerrak stations, but less so than at the deep Porcupine Abyssal Plain (4850 m).

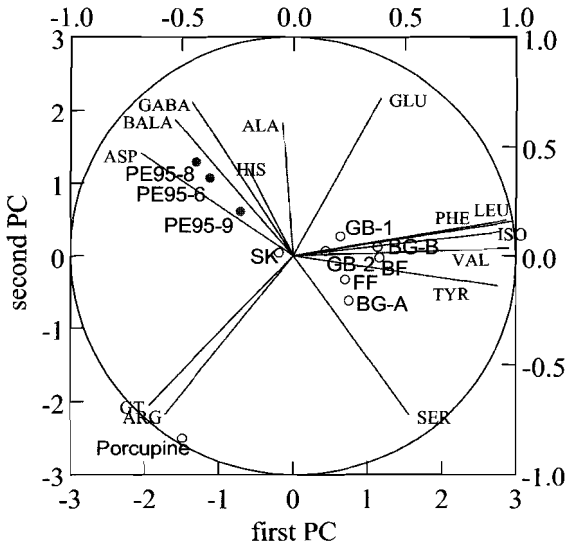

Figure 2.8 Results of a PCA applied to normalised amino acid mole% from sediments of the Goban Spur continental slope (●) (651 m, 1296 m and 3650 m; shown by solid circles) sediments of seven North Sea stations ranging from relatively fresh to refractory (○) (BG-B, BF, FF, BG-A, GB-1, GB-2 and SK; abbreviations explained in table 3; shown by open circles) and sediment of the Porcupine Abyssal Plain (○) (Porcupine; see table 3; shown by open circle). Coefficients for standardised factor scores on the first and second PC are given for the individual amino acids. Abbreviations of the amino acids were explained in fig. 2.3.

A second PCA was applied to normalised mole% of amino acids in the top layer sediment size fractions. The highest site score along the first PC was calculated in the finest size fraction at 651 m (Table 2.3). Site-scores of the coarse fraction at 651 m and all size fractions at the deeper stations showed little variation. There was a good correlation between mole% of the labile phenylalanine, isoleucine and leucine with site-scores on the first PC (Fig. 2.9B), which were used as indicators for OM degradation state by Dauwe and Middelburg (1998) and Dauwe et al. (1999). The OM in the finest and to a lesser degree in the two following intermediate fractions at 651 m appeared to be considerably less degraded than in the coarsest fraction or any size fractions at the deeper stations.

Table 2.3 Results of PCA, applied to normalised amino acid mole% of sediments from the Goban Spur continental slope, the North Sea and Porcupine Abyssal Plain. Methionine was not detected in the sediments. Lysine was not included in the PCA. Data of the North Sea stations were taken from Dauwe et al. (1998); SK: Skagerrak, GB-2: German Bight (1-15cm), GB-1: German Bight (0-1 cm), BG-A and BG-B: Brouwershavensche Gat A and B, FF: Frisian Front, BF: Broad Fourteens. Data of the Porcupine (station PAP; 4850 m) were taken from Horsfall and Wolff (1997). A second PCA was applied to normalised amino acid mole% in sediment size fractions.

	factor coefficients	r^2 with 1st PC	r^2 with 2nd PC		Site scores
PCA 1.					
aspartic acid	-0.102	0.46	0.22	SK	-0.190
glutamic acid	0.059	0.16	0.52	GB-2	0.437
Serine	0.078	0.27	0.53	GB-1	0.636
G+T	-0.096	0.41	0.46	BG-A	0.754
Alanine	-0.007	0.00	0.37	FF	0.696
Tyrosine	0.137	0.84	0.02	BF	1.158
Metionine	n.d.	n.d.	n.d.	BG-B	1.134
valine	0.145	0.94	0.00		
phenylalanine	0.147	0.96	0.02	PE95-9	-0.709
isoleucine	0.139	0.86	0.01	PE95-6	-1.122
leucine	0.143	0.91	0.03	PE95-8	-1.306
lysine	n.a.	n.a.	n.a.		
histidine	-0.03	0.04	0.16	Porcupine	-1.488
arginine	-0.086	0.33	0.52		
ß-alanine	-0.079	0.28	0.39		
γ-aminobutyric acid	-0.068	0.21	0.50		
	factor coefficients	r^2 with 1st PC			Site scores
PCA 2.					
aspartic acid	-0.114	0.72	PE95-9	<0.5µm	2.268
glutamic acid	-0.029	0.05		0.5-2µm	0.702
serine	-0.001	0.00		2-10µm	1.575
G+T	-0.11	0.66		>10µm	-0.371
alanine	0.108	0.64			
tyrosine	0.07	0.27	PE95-6	<0.5µm	-0.529
methionine	n.d.	n.d.		0.5-2µm	-0.500
valine	0.107	0.63		2-10µm	-0.080
phenylalanine	0.125	0.86		>10µm	-0.772
isoleucine	0.126	0.87			
leucine	0.123	0.83	PE95-8	<0.5µm	-0.836
lysine	0.076	0.32		0.5-2µm	-0.850
histidine	-0.077	0.33		2-10µm	-0.355
arginine	0.11	0.67		>10µm	-0.252
ß-alanine	-0.069	0.26			
γ-aminobutyric acid	-0.076	0.32			

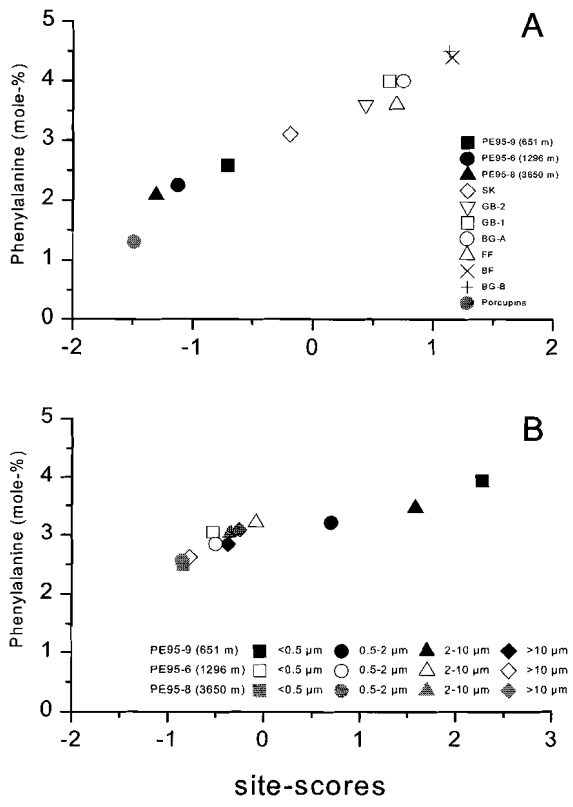

Figure 2.9 (A) Mole% of phenylalanine plotted against site-scores on the first PC from sediments of the Goban Spur continental slope, seven North Sea stations and the Porcupine Abyssal Plain. Abbreviations of the stations are given in Table 3. (B) Mole% of phenylalanine plotted against site-scores on the first PC of the sediment size fractions of top layer sediments (0-2.5 mm) from PE95-9 (651 m), PE95-6 (1296 m) and PE95-8 (3650 m).

Summarising, we conclude that (aggregates of) small particles (<0.5 µm) with high THAA concentrations and high reactivity are deposited initially at the upper slope (651 m). The reduced amount of fine particles in the top layer relative to deeper layers at the upper slope and an increasing contribution of fine particles at the deeper stations point to erosion at the upper slope and subsequent down slope transport, sorting and accumulation of fine particles. These processes could explain the relatively high TOC and THAA concentrations in the sediment top layer at the deeper stations. Continuous mineralisation of OM attached to the particles during vertical and lateral transport results in an increasing organic matter degradation state from the upper slope to the abyssal plain.

3.

Mid-slope accumulation of amino acid-rich organic matter across the Faeroe-Shetland Channel.

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Abstract

In the framework of the PROCS project (PROcesses on the Continental Slope), we present data on total organic carbon (TOC), total nitrogen (TN) and total hydrolysable amino acids (THAA) in samples of suspended matter (SM), sediment traps and surface sediments across the southeastern slope of the Faeroe-Shetland Channel. Aim of the study was to examine whether sediment resuspension and down slope transport results in cross slope variations of amino acid concentrations in surface sediments. The TOC, TN and THAA concentration in suspended matter generally decreased with depth in the water column and from the shelf-break towards the center of the channel. However, the contribution of amino acid-nitrogen to TN (%-AAN) in suspended matter increased with water depth and indicated that aggregates settling from the surface layer are not the dominant source of organic matter in near-bottom water layers. In near-bottom sediment traps, THAA concentrations and amino acid distributions mostly resembled that of near-bottom SM. A simple end-member model demonstrated that near-bottom SM consists of ~80% of fine particles (<63 μm) originating from the sediment surface that are semi-permanently in suspension and that the primary flux from the upper water column contributes only to ~20%. A Principal Component Analysis, applied to amino acid distributions in the samples, suggested that particles were eroded from the upper slope and transported down slope in near-bottom SM layers. The mid-slope maximum of fine particles in the surface sediment as well as elevated inventories of ^{234}Th and high values of %-AAN evidenced the deposition of young and highly reactive organic matter at this depth. It is concluded that cross-slope variations in amino acids were caused by down slope transport of fine, amino acid-rich particles that preferentially settle at mid-slope depth.

Introduction

The vertical flux of organic matter (OM) through the water column is dominated by large, rapidly settling aggregates (Honjo et al. 1982b; Fowler and Knauer, 1986). Newly formed aggregates are rich in labile organic matter and are, therefore, active sites of mineralisation (Cho and Azam, 1988; Alldredge and Gotschalk, 1990). In shelf seas of high primary productivity as much as 10-15% of the annual water column OM production can reach the sediments (Lee and Cronin, 1982), whereas in the open ocean this is only 1-3% (Suess, 1980; Jahnke, 1996; Lampitt and Antia, 1997; Romankevich et al., 1999).

Biscaye and Anderson (1994) found that at the shelf-break off the NE American coast (Middle Atlantic Bight) the particulate matter flux in deeper sediment traps was a

factor 5-25 higher than the flux in shallow traps. Antia et al. (1999) reported that at the NE Atlantic continental slope (Goban Spur) the POC flux in deeper traps was more than a factor two higher than in the upper water column, which they explained by strong lateral input. This increase of the POC flux with depth in the water column originates from particles in nepheloid layers (McCave, 1986). Nepheloid layers can protrude laterally from the shelf-break into the interior of the ocean (Dickson and McCave, 1986), or follow the continental slope in bottom nepheloid layers (BNL) (McCave, 1986). Repetitive cycles of resuspension, disaggregation-aggregation and transport of particles in the BNL hydrodynamically sorts small OM-rich from coarse OM-poor particles (Thomsen, 1999). The intensity of the BNL, which is strongest at the continental shelves and slopes, depends on the strength of the bottom currents (McCave, 1986), internal wave activity (Dickson and McCave, 1986) and the sediment bed structure (Thomsen, 1999). Therefore, in slope areas with strong currents and internal wave activity the down slope transport of particles can be a major mechanism for OM deposition to the sediments in addition to direct vertical settling from the euphotic zone.

During the pilot study of the PROCS project 1997 (PROcesses on the Continental Slope) in the Faeroe-Shetland Channel (FSC) the interactions were studied between bottom topography, the occurrence and distribution of internal waves and the associated effects for sediment resuspension and cross slope zonation of organic matter in sediments. Van Raaphorst et al. (2001) reported that internal waves of tidal frequency could be responsible for sediment erosion at depths shallower than 550 m and deposition at depths deeper than 600 m, particularly between ~700-800 m on the slope. From near-bottom current velocities and from total mass fluxes (TMF), total organic carbon (TOC) and total nitrogen (TN) in near-bottom sediment traps, Bonnin et al. (submitted) calculated that during events of high current velocities trapped particles were a mixture of fluffy aggregates from the BNL and coarse sediment particles (low TOC and TN). The high current velocities corresponded to high TMF, which indicated resuspension from the seabed. According to their results, there was only a minor contribution of particles settling directly from the upper water column to the total flux and a major contribution from sediment resuspension.

Here, we present data on total hydrolysable amino acids (THAA) in suspended matter, sediment traps and surface sediments from the PROCS 99-1 cruise (April-May 1999) to further evaluate the cross slope variations of OM and the associated effects for OM degradability. Since the contribution of amino acid-carbon to TOC in organic matter decreases during diagenesis and since the distribution of individual amino acids to THAA changes as a result of preferential mineralisation as well as by adsorption onto clay minerals and carbonates (Henrichs et al., 1984; Lee, 1988; Sugai and Henrichs, 1992; Cowie and Hedges, 1992b; Nguyen and Harvey, 1997; Boski et al., 1998) amino acids are a sensitive tool to study OM degradability (Dauwe and Middelburg, 1998; Grutters et al., 2001a). Firstly, suspended matter samples are discussed to characterize particles with depth in the water column. Secondly, near-bottom sediment trap samples are discussed, in combination with data from Bonnin et al. (submitted), to qualify as well as to quantify the resuspension flux of amino acids across the slope. Then, data on grain-size analysis, ^{234}Th, TOC, TN and THAA in the sediments are combined to evaluate cross slope erosion-deposition patterns of amino acids in the sediments. A Principal Component Analysis

(PCA) was applied to normalized amino acid mole-percentages to assess the OM degradability across the slope.

Material and Methods

Study Area-The Faeroe-Shetland Channel (60°N, 6°W-63°N, 1°W) connects the Norwegian Sea with the Atlantic Ocean. It has a maximum depth of 1500-2000 m at the northeastern entrance and is about 600-650 m deep at the Wyville-Thomson Ridge in the south (Fig. 3.1). The upper 200-500 m of the water column is composed of two distinct water masses, North Atlantic Water (NAW) flowing northward along the West Shetland shelf and Modified North Atlantic Water (MNAW) flowing southward across the Faeroe Shelf and upper northwestern slope. This MNAW turns in front of the Wyville-Thomson ridge and then runs northward parallel to the NAW along the central axis of the Channel. Deep water (>500 m; FSCBW) comes from the Norwegian Basin and flows southward towards the Wyville-Thomson Ridge and further. The morphology and hydrography of the margin are described in detail by Stoker et al. (1993) and Turrell et al. (1999).

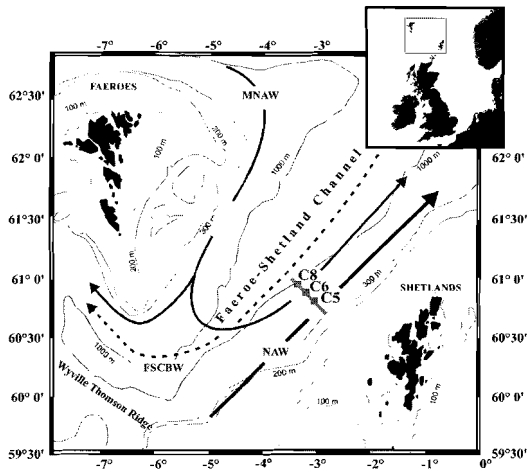

Figure 3.1 Location of sampling stations across the slope of the Faeroe-Shetland Channel. Sediment trap samples (□) were obtained from moorings C5 (700 m), C6 (800 m), and C8 (1000 m). The solid line through the mooring sites represents the transect where suspended matter and sediment samples were collected. Sediment samples were obtained from station 78 (300 m), 11 (370 m), 61 (550 m), 54 (670 m), 71 (750 m), 68 (850 m) and 128 (900 m). Suspended matter samples were taken at the position of station 78, 61, 68 and 128 at water depths of 10 m (surface), 250 m and 10 mab (near-bottom nepheloid layer).

Samples-Suspended matter, sediment trap, and surface sediment samples were collected along a trajectory from 60.73°N, 2.87°W (300 m water depth) to 61.00°N, 3.18°W (1000 m water depth) across the south-eastern slope of the Faeroe-Shetland Channel with R.V. *Pelagia* during the PROCS 99-1 cruise from 14 April - 5 May 1999. Sediment trap data of this cruise are reported by Bonnin et al. (2001).

Suspended matter samples were collected at stations 78, 61, 68, 128 (Fig. 3.1) across the slope with a CTD-Rosette system containing a Seabird 911 CTD. The rosette holds 22 12-l NOEX bottles. An amount of 40 liters was collected from the surface waters, 250 m water depth, and 10 meters above the seabed (mab). In a thermostated laboratory at ambient bottom water temperature the water was filtrated through precombusted Whatman GFF filters (0.7 μm). Filters were stored frozen (-20°C) until analysis at the NIOZ.

Moorings C5, C6, and C8 (Fig. 3.1) consisted of a buoyancy package at the top and a weight at the bottom coupled to two acoustic releases. The sediment traps were located at 2 mab and 30 mab. Moorings C5 and C6 were equipped with PPS 4/3-type traps with 12 sampling cups. Mooring C8 contained an HDW-type trap in the bottom frame with 20 collecting cups. The traps were synchronized to collect samples at 12 intervals of 24 hours, from 18-30 April. In order to collect samples in a high current regime (Butman et al., 1986) all traps were modified to similar aspect ratios of 8 by elongating the trap to a total height of 2 m with PVC cylindrical tubes covered with a baffled screen (10 mm hexagons). The collecting area was 0.04 m^2 (∅=25 cm). Prior to deployment all collecting cups were filled with seawater from the deployment site at the specific depths. A biocide (0.50 g l^{-1} $HgCl_2$) and a pH buffer (2 mg l^{-1} $Na_2B_4O_7.10H_2O$) were added to the cups to minimize organic matter degradation and carbonate dissolution. NaCl was added to bring the solution in the sampling cup to seawater density. After retrieving of the moorings, the sediment trap cups were stored at 4°C until further processing at the NIOZ. There, the sediment trap samples were filtrated over 0.4 μm polycarbonate filters (47 mm diameter), washed with 2 ml of MilliQ water and freeze-dried. After freeze-drying the sediment material was carefully removed from the polycarbonate filters and stored in clean glass vials with PTFE-lined caps until analysis (Bonnin et al., 2001).

Sediment samples were collected with a box-corer or a multi-corer at stations 78, 11, 61, 54, 71, 68, and 128 (Fig. 3.1) and processed at ambient temperature directly upon retrieval. Cores were sliced into intervals of 4x2.5 mm (0-1 cm). 4x5 mm (1-3 cm), 4x10 mm (3-7 cm), and 4x20 mm (7-15 cm), using the high-precision hydraulic core-slicer as described in Van der Zee et al. (2001). The sediment slices were centrifuged (3000 rpm, 10 min) for pore water extrusion and freeze-dried. The freeze-dried samples were homogenized and ground by an automated tungsten mortar and kept in clean glass vials with PTFE-lined caps until analysis.

Sediment size fractions-Splits of the sediment top layer of all stations were fractionated into three size fractions (<63 μm, 63-250 μm and >250μm) by dry sieving. Approximately 1-2 g of freeze-dried material was used for sieving. The size fractions were checked microscopically for the presence of aggregates. The coarser size fractions (63-250 μm and >250 μm) did not contain aggregates of smaller particles and/or faecal pellets after ~5 minutes of intensive sieving. The fractionated samples were kept in clean glass vials with PTFE-lined caps until analysis.

Particle size analysis-Particle size was measured with a Coulter LS230 laser particle-sizer as described by Konert and Vandenberghe (1997). Approximately 500 mg of freeze-dried material was suspended in ~10 ml water and ultra-sonicated for 15 minutes. Before putting the sonicated suspension into the Coulter, the suspension was sieved over 1 mm. The suspensions in the Coulter were diluted to an obscuration of 10% before the measurements

were performed. During the measurements all samples were ultra-sonicated internally. Grain size distributions were corrected for the weight retained on the 1 mm sieve.

^{234}Th activity-The ^{234}Th activity was measured directly after the cruise in 15 g of ground freeze-dried samples either by counting the 92 keV gamma emission with a high-resolution planar germanium GC3019 detector or by counting the 63 keV and 92 keV gamma emission with a low energy GL1020R detector after Buesseler et al. (1992). Samples from a single core were measured with the same detector. Calibration was done with an external ^{238}U standard in a silicate matrix. Supported activity was measured after four months. Excess ^{234}Th activity, the difference between the initial count and the supported activity, was corrected for the time elapsed between sample collection and counting.

Total organic carbon (TOC) and total nitrogen (TN)-Filtrated, freeze-dried suspended matter samples as well as freeze-dried, homogenized and ground sediment trap and sediment samples were analyzed for TOC and TN on a Carlo-Erba 1500 elemental analyzer, following the procedure of Verardo et al. (1990) and modified after Lohse et al. (1998, 2000). The amount of calcium carbonate was derived from the difference between total carbon (non-acidified) and TOC (after acidifying with sulphurous acid).

Amino acids-Samples of suspended matter, sediment traps and sediments were analyzed for amino acids by reverse-phase HPLC analysis as described by Lindroth and Mopper (1979), Cowie and Hedges (1992a) and modified after Dauwe and Middelburg (1998). Approximately 150 mg of freeze-dried sediment was weighed in pre-combusted 8-ml glass vials with PTFE lined screw caps. An amount of 5 ml HCl (6 M) was added to each vial. The samples were flushed with N_2 for at least three minutes and the vials were closed under nitrogen atmosphere. Samples were then hydrolyzed at 110°C for 24 hours. Hydrolysates were filtered through 0.45 µm Gelman syringe filters (low protein binding). A subsample of 50 µl was pipetted into sterile 2 ml glass vials and dried under vacuum. The dried samples were washed in 500 µl of MilliQ water and dried again under vacuum three times to remove all traces of acid. Finally, the samples were dissolved in acetic acid buffer (pH 7.0) and kept frozen until analysis. If necessary, the samples were further diluted with acetic acid buffer to adjust the concentration to the concentration range of the amino acid standards (150-1500 nM).

Prior to HPLC analysis, fluorescent derivatives of the amino acids were formed by pre-column reaction with *o*-Phtaldialdehyde (OPA) (Lindroth and Mopper, 1979), buffered to a pH of 9.5. The HPLC system consisted of a Hewlett Packard series 1050 pump and auto-injector and a Hewlett Packard 1046A fluorescence detector. A Chromsep SS reverse-phase column (250x4.6 mm) with Chromsep SS reverse phase guard columns (10x3 mm) and a binary solvent system were used to separate seventeen amino acids during a run of 40 minutes. A description of the binary solvent system, the solvent gradient and the detection has been given in Grutters et al. (2001a).

Concentrations of the amino acids were calculated from peak areas, which were calibrated with a standard amino acid mixture (AA-S-18, SIGMA chemicals) ranging in concentration from 150 to 1500 nM. β-Alanine (PAA-11, SIGMA chemicals) and γ-aminobutyric acid (PAA-9, SIGMA chemicals), were added as standards for the non-protein amino acids. No internal standards were used to account for losses that may have

occurred during hydrolysis or analysis. Peak areas were corrected for procedural blanks before calculating sample concentrations. The reproducibility of the analysis, determined from triplicate injections, was better than 2% (expressed as CV) for all amino acids, except histidine, methionine and tyrosine (3-4%). For all amino acids, the correlation between peak areas and standard concentration was good (r^2>0.95). The precision of duplicate samples was 10-15% for all amino acids, except γ-aminobutyric acid (17%) and tyrosine (19%).

Results

Suspended matter in the water column

The suspended matter concentration varied between 0.4 mg l^{-1} and 1.0 mg l^{-1} without a clear trend with water depth (shown as values between brackets in Fig. 3.2a).

TOC (Fig. 3.2a), TN (Fig. 3.2b) and THAA (Fig. 3.2c) content in the particles generally decreased with increasing water depth at all stations. In the euphotic layer the concentrations decreased laterally towards the center of the FSC. Deeper in the water column (250 m depth and near-bottom), the concentrations first decreased off shelf but increased again at the deepest part of the FSC.

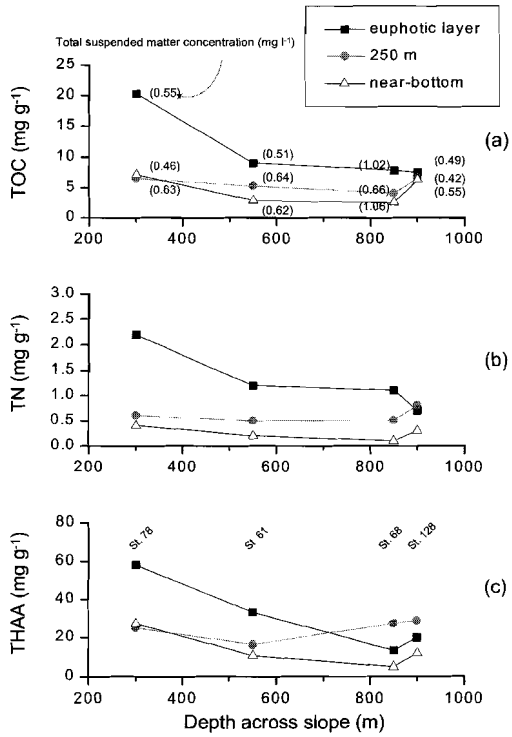

Figure 3.2 TOC concentrations (a), TN concentrations (b) and THAA concentration (c) in suspended matter samples from stations 78 (300 m), 61 (550 m), 68 (850 m) and 128 (900 m). Suspended matter samples were taken in the euphotic layer (~10 m), 250 m and near the sea floor. Total suspended matter concentrations are shown in brackets in panel (a).

The amino acid distributions in suspended matter, given as mole-percentages, are given in Fig. 3.3. Glycine, glutamic acid, aspartic acid and alanine were the most abundant amino acids, accounting for 40-48% of the total amount of THAA. The variation in amino acid distributions in vertical direction (with increasing water depth) as well as in lateral direction (between the stations across the slope) was small. Glycine, however, was slightly enriched at all depths in the water column at station 128 (900 m). Alanine was relatively depleted at station 78 (300 m) and in the surface water of station 68 (850). Lysine was relatively enriched in the surface water at station 78 and at all depths at station 68.

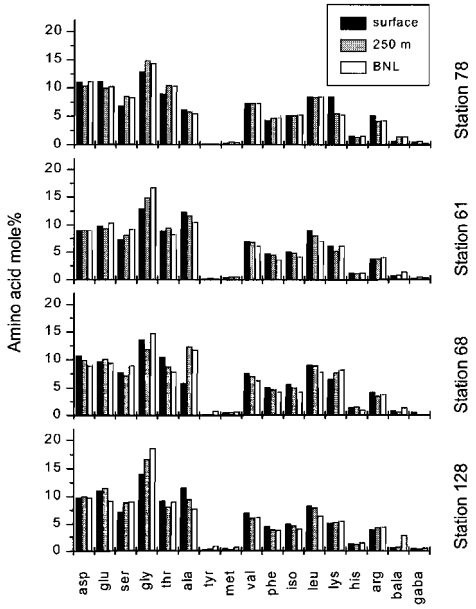

Figure 3.3 Amino acid distributions in suspended matter at stations 78 (300 m), 61 (550 m), 68 (850 m) and 128 (900 m). The black, gray and white bars denote the samples from respectively the surface, 250 m depth and near-bottom layers. The abbreviations stand for aspartic acid (asp), glutamic acid (glu), serine (ser), glycine (gly), threonine (thr), alanine (ala), tyrosine (tyr), methionine (met), valine (val), phenylalanine (phe), iso-leucine (iso), leucine (leu), lysine (lys), histidine (his), arginine (arg), β-alanine (bala) and γ-amino-n-butyric acid (gaba).

Sediment traps

Total mass flux (TMF), TOC and TN in sediment trap samples from the moorings C5 (700 m), C6 (800 m) and C8 (1000 m) were described in detail by Bonnin et al. (2001). At C5 and C6 TMF sharply increased at yearday 112 (Fig. 3.4), and decreased again to its background value after yearday 115. At C8 the TMF was low during the whole trapping period. For C5 and C6, samples taken from day 112 to day 115 will be referred to as high flux samples. The samples from the remaining days as well as the samples from C8 will be referred to as low flux samples.

Here, we would like to focus on the relative differences between periods of high and low TMF. For each period we will present TOC and TN concentrations that are averages of the two traps that are attached to each mooring (Table 1). For a more extensive description of TOC and TN values in the traps we refer to Bonnin et al. (2001). TOC concentrations in the trap samples were lower during periods of high TMF (day 112-115)

than during periods of low TMF (day 108-111; 116-119) (Fig. 3.4). At C8, where low TMF were detected only, the TOC concentration was always close to that of the low flux samples of C5 and C6. TN and THAA concentrations were also low during high flux periods and higher during low flux periods (Table 1).

The differences in amino acid distributions in the traps were small between periods of high and low fluxes (Fig. 3.5). Also, the differences between the traps from different mooring depths were small. In all samples glycine, aspartic acid, alanine, glutamic acid and serine were the most abundant amino acids, accounting for 57-60% of the THAA.

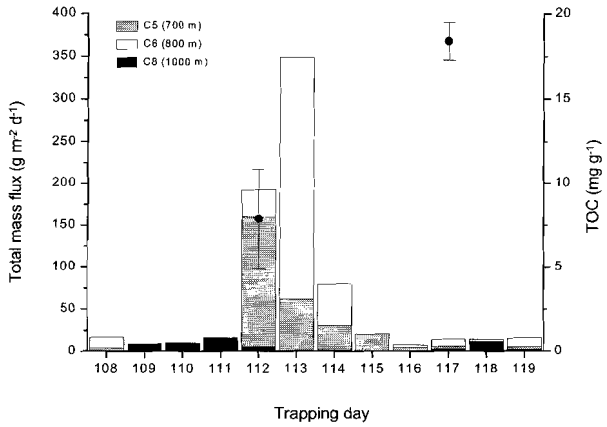

Figure 3.4 Total mass fluxes in the sediment traps at moorings C5 (700 m), C6 (800 m) and C8 (1000 m). Here, we show only fluxes for the trap just above the seafloor (2 mab). For a more detailed description of mass fluxes in the sediment traps, we refer to Bonnin et al. (2001). The sampling period was 12 days. The period between yearday 112 and 115 is referred to as high flux period, whereas the remaining period (day 108-111, 116-119) is referred to as low flux period. Also shown is the TOC concentration in a high and low flux sample (●) from mooring C5. These TOC concentrations are average values of the two traps that are attached to each mooring (2 mab and 30 mab) and demonstrate that the TOC concentration in the trap samples is higher during low flux periods than during high flux periods.

Table 3.1 Total mass flux, TOC, TN, THAA concentrations and %AA-N values for the sediment traps at moorings C5 (700 m), C6 (800 m) and C8 (900 m). These values are average values for the two traps attached to each mooring (2 mab and 30 mab). For TOC, TN and THAA the standard deviation for the average concentrations between the two traps is shown.

	Period	Mass Flux (g m^2 d^{-1})	TOC (mg g^{-1})	TN (mg g^{-1})	THAA (mg g^{-1})	%-AAN
C5	High flux	85.4	7.9±3.0	1.0±0.4	3.0±1.3	48.0
C5	Low flux	4.6	18.4±1.1	2.5±0.1	8.6±2.4	51.3
C6	High flux	186.3	7.6±3.3	1.0±0.5	3.2±1.6	54.6
C6	Low flux	14.4	18.4	2.5	9.1	49.5
C8	Low flux	5.6	18.3±1.0	2.7±0.2	9.4±1.2	54.2

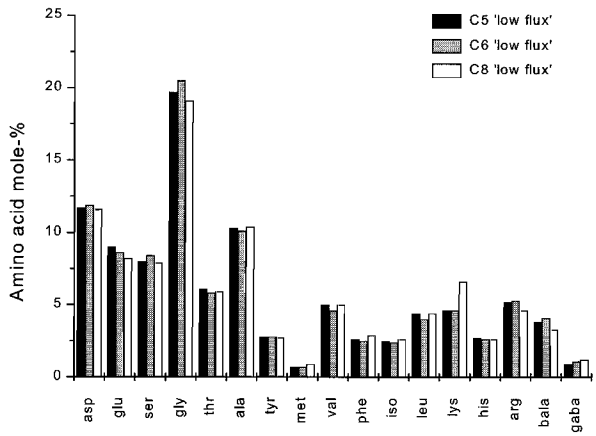

Figure 3.5 Amino acid distributions in sediment trap samples from low flux events at moorings C5 (700 m), C6 (800 m) and C8 (1000 m). The amino acid distributions are averages of the two traps attached to the moorings (2 mab and 30 mab). The amino acid distributions in the sediment trap samples from high flux events are very similar to the distributions from low flux events and are, therefore, not shown here. Abbreviations for the amino acids were explained in Figure 3.2.

Sediments

Visual inspection of the sediment cores showed that at stations 78 and 11 (upper slope) the sediment consisted of brown colored clays that were overlain by ~5 cm of grayish-brown coarse sand. At station 71 (mid slope), clay was not present in the upper 10 cm, and at stations 68 and 128 (lower slope) clay was observed only deeper than 9 cm. The transition from clay to coarse sands was interpreted as the changeover from glacial to interglacial conditions (Stoker et al., 1991) and illustrates that since the early Holocene sediment accumulation was stronger at the lower than at the upper slope. The visual observations of sediment texture corresponded with the median grain sizes across the slope. At the shallow stations the median grain size was high in the upper ~3 cm and decreased further down core (Fig. 3.6). At the deeper stations the median grain size was rather constant (~150 μm) with sediment depth. The average median grain size for the upper ~3 cm of the sediment decreased from ~400 μm at the upper slope to ~150 μm at the lower slope, which is close to the values measured during the PROCS 97 pilot study (sediments deeper than 550 m, Van Raaphorst et al., 2001).

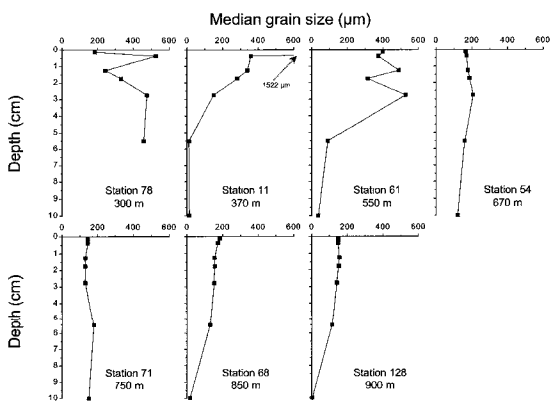

Figure 3.6 Median grain size in the upper 10 cm of the sediments across the Faeroe-Shetland Channel. At station 78 m (300 m) the median grain size was not measured at 10 cm depth. At station 11 (370 m) the median grain size of 1522 μm in the sediment surface was out of scale.

The weight percentages of the size fractions <63μm, 63-250 μm and >250 μm are shown in Fig. 3.7 for station 11 (370 m), 71 (550 m) and 128 (900 m). At the shallow station 11 the fraction <63 μm increases with sediment depth, the >250 μm fraction decreases with depth. At the mid-slope station 71 the distributions are more or less constant with depth, with the 63-250 μm fraction being the most abundant. At the deepest station 128 the <63 μm fraction increases with depth to almost 100%, with a complementary decrease of the coarser fractions. The increasing contribution of the <63 μm fraction with sediment depth at stations 11 and 128 corresponds to the visually observed clay underlying the sand layer (Fig. 3.7).

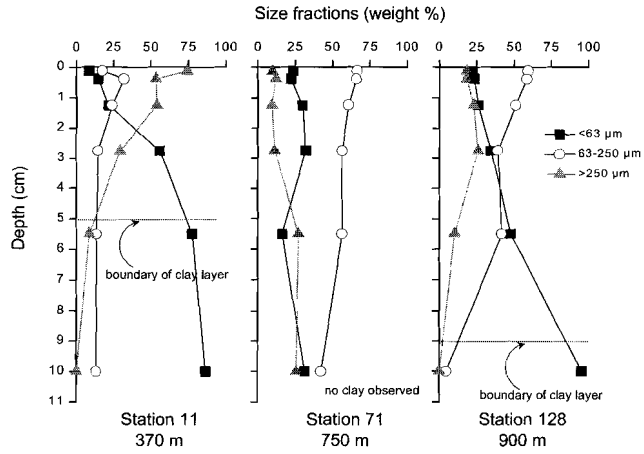

Figure 3.7 Sediment size fractions in weight-percentages, as obtained from particle size analysis, in the upper 10 cm of sediments at station 11, 71 and 128 across the FSC. Here, only the cumulative weight percentages for the <63 μm, 63-250 μm and >250 μm fractions were shown. The dashed lines denote the transition from the sand to clay layer, based on the increase of the fraction <63 μm as well as on visual inspection of the sediment cores.

^{234}Th profiles reached their background concentration within the upper centimeter of the sediments at all stations. The inventory of ^{234}Th in the upper centimeter increased almost five-fold from 250 mBq cm^{-2} at 300 m to almost 1200 mBq cm^{-2} at 750 m and to ~700 mBq cm^{-2} at 850 and 900 m depth (Fig. 3.8a).

The TOC content in the surface sediment decreased from 6.5 mg g^{-1} at station 78 (300 m) to 2.9 mg g^{-1} at station 11 (370 m) and was more or less constant further down slope (Fig. 3.8b). At station 11 the TOC concentration decreased from the sediment surface to 12.5 mm and increased further down core (Fig. 3.9). At station 71 (750 m) the TOC concentration increased from the surface to a maximum at ~1 cm and then decreased further down core. The TOC concentration at station 128 (900 m) was more or less constant in the upper ~2 cm and then decreased down core.

The highest calcium carbonate concentration was found at the upper slope (47%) and decreased to 10% at the lower slope (Fig. 3.8c). The calcium carbonate concentration was invariant with sediment depth, except at station 11 where the carbonate concentration decreased from ~39% at the sediment surface to 12% at 10 cm depth.

The THAA concentration at the sediment surface varied between 0.7-2.0 mg g^{-1} across the slope (Fig. 3.8e). THAA concentrations in the 63-250 μm and >250 μm fraction were very

similar to those in the bulk sediment. Apart from station 11, the THAA concentration in the finest fraction was 3-5 times higher than in the bulk sediment. The THAA concentrations with sediment depth were rather constant in the upper 12.5 mm and then decreased down core, except at station 11 where the profile showed a sub-surface maximum (Fig. 3.9).

In Fig. 3.10 amino acid distributions with sediment depth are given for station 71 (750 m). Aspartic acid, alanine and glycine increased with sediment depth, which was most pronounced for glycine. Serine, phenylalanine, isoleucine and leucine decreased with sediment depth. The amino acid distributions at the other stations across the slope were very similar to station 71 and are, therefore, not shown.

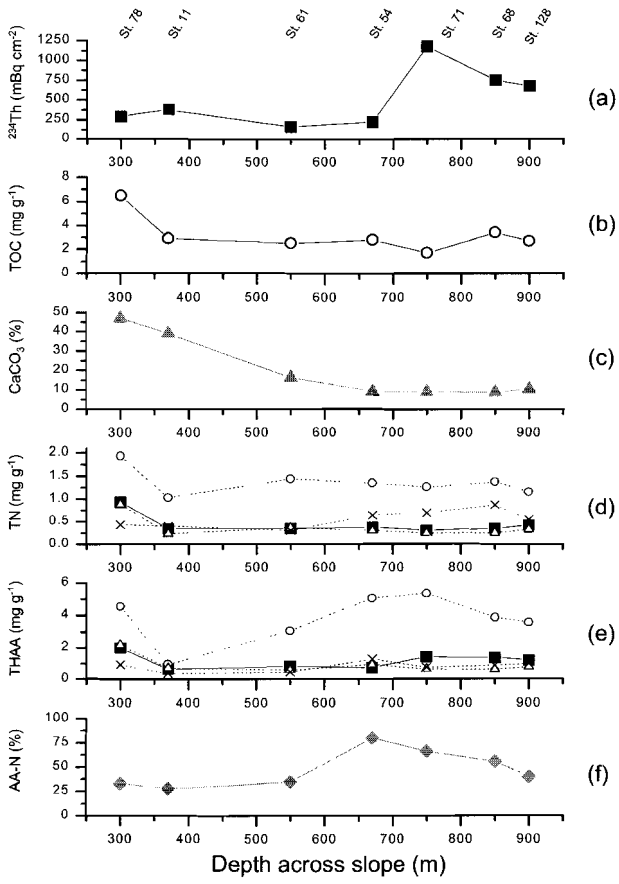

Figure 3.8 ^{234}Th inventories were determined in the upper ~1 cm of the sediment at station 78, 11, 61, 54, 71, 68 and 128 (a). TOC was measured in the surface sediment (0-2.5 mm) after acidification of the samples with sulphurous acid (b). The difference between total carbon and TOC represents the fraction of calcium carbonate (c) in the samples. TN was analyzed in the surface sediment (0-2.5 mm), in bulk sediment (■) as well as in the <63µm fraction (●), the 63-250 fraction (▲) and the >250 fraction (×) (d). THAA was analyzed in the surface sediment (0-2.5 mm), in bulk sediment (■) as well as in the <63µm fraction (●), the 63-250 fraction (▲) and the >250 fraction (×) (e). The contribution of amino acid-nitrogen to total nitrogen (%AA-N) in the sediment surface (0-2.5 mm) is shown in panel (f).

The nitrogen content in the bulk sediment decreased from 0.9 mg g^{-1} in the surface sediment of station 78 (300 m) to 0.3 mg g^{-1} at station 11 (370 m) and then remained more or less constant further down slope (Fig. 3.8d). TN concentrations were also measured in the size fractions from the sediment top layer. The TN concentration in the finest fraction was 2-3 times higher than TN concentrations in the coarse size fractions at all stations.

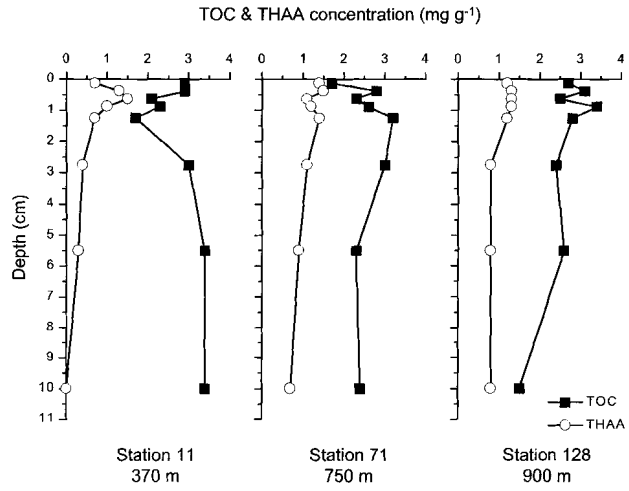

Figure 3.9 TOC (■) and THAA (○) concentrations in the upper 10 cm of sediments at stations 11 (370 m), 71 (750 m) and 128 (900 m) across the FSC slope.

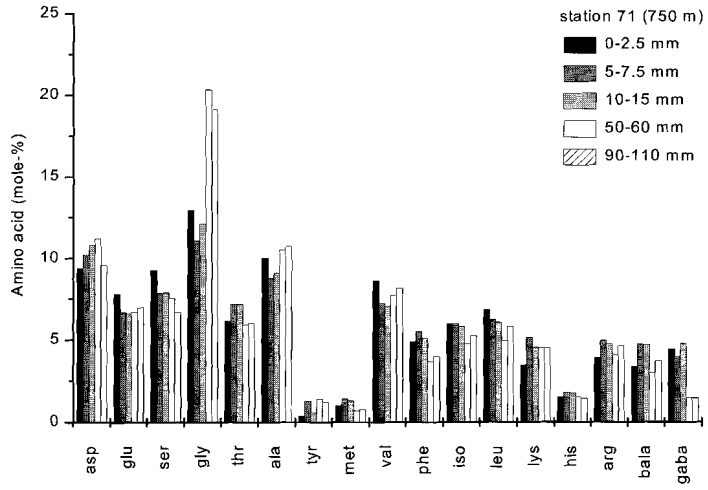

Figure 3.10 Amino acid distributions with sediment depth at station 71 (750 m). The slice thickness is shown in the legend; the amino acid distributions are averages for each slice. Abbreviations for the amino acids were explained in figure 3.2.

Discussion

In the open ocean, fluxes of total mass as well as of POC generally decrease with increasing water depth (Suess, 1980; Jahnke, 1996; Lampitt and Antia, 1997). However, several studies demonstrated that near continental slopes total mass fluxes and fluxes of POC can increase with water depth (Honjo et al., 1982a; Antia et al., 1999), suggesting lateral input (Jahnke, 1990; Biscaye and Anderson, 1994). Bottom nepheloid layers (BNL) are considered as an important mechanism for down slope transport of particles (McCave, 1986; Thomsen, 1999). Repetitive cycles of resuspension-deposition and disaggregation-aggregation of freshly deposited aggregates can stimulate bacterial activity and degradation of organic matter. (Turley and Lochte, 1990; Ritzrau, 1996; Boetius et al., 2000a; Nagata et al., 2000). Thus, the organic matter degradability will decrease due to ongoing, possibly enhanced, microbial attack during the near-bottom down slope transport. This lateral transport in nepheloid layers adds to the classical mechanism that the OM degradability in ocean sediments decreases with increasing water depth due to increased exposure time of aggregates to mineralisation during settling in the water column (Suess, 1980; Lee et al., 1998; Lampitt and Antia, 1997). The combined mechanisms are nicely exemplified by Epping et al. (2001) for the Iberian Margin.

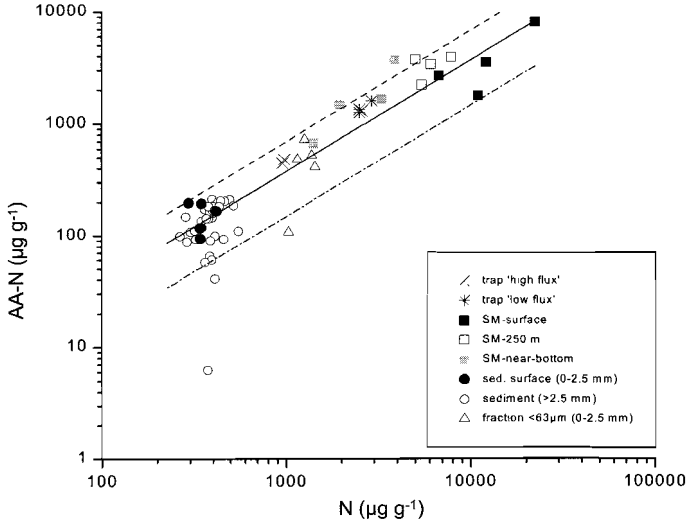

Figure 3.11 The relationship between amino acid-nitrogen and total nitrogen in suspended matter (SM) samples from the water column (■), sediment traps (×) and bulk sediment (●) as well as in the finest sediment size fraction (▲). The lines denote different ratios of amino acid-nitrogen to total nitrogen (%AA-N) for the range of TN concentrations. The area between the fixed %AA-N of 70% (dashed) and 38% (solid) represents the fresh OM, whereas the area between %AA-N of 38% and 15% (dash-dotted) represents the degraded OM.

The contribution of amino acid-nitrogen to TN is an indicator of organic matter degradability. Preferential utilization of amino acids causes the contribution of amino acid nitrogen to TN to decline from 75-90% in fresh plankton to 40-50% in OM in sinking aggregates to 10-30% in sedimentary OM (Lee, 1988). In the FSC, TN concentration in the

suspended matter samples decreased from the ocean surface to near the seafloor, while the amino acid-N concentration was more or less constant with water depth (Fig. 3.11). Consequently, the %AA-N increased towards the seafloor, which is in contrast with the general expectation. This suggests that close to the seafloor suspended matter mixes with amino acid-rich organic matter, either from the surface sediment or from near-bottom secondary production. Fig. 3.11 shows that when comparing near-bottom suspended matter with trap samples and then with the sediment top layer, the TN and AA-N concentrations proportionally decrease, suggesting that there is no preferential degradation of amino acids near the sea floor. However, the deeper sediment samples all have similar TN concentrations but lower AA-N values than at the sediment surface indicating that upon burial the amino acids become preferentially degraded.

Bonnin et al. (2001) observed that during periods of low fluxes (i.e. low resuspension) the material in the traps consisted almost completely of 'fluffy' particles associated with the BNL and the sediment surface, and that there was only a marginal contribution of particles settling from the upper water column. From a simple end-member model, based on TOC concentrations, they estimated that during periods of high mass flux (i.e. increased resuspension) the sediment trap material became diluted with coarse particles from the surface sediment.

Here, we will further evaluate the contribution of particles from the upper water column and from near-bottom layers to the particles in the sediment traps. We used THAA and TN concentrations as well as mole-percentages of some of the most abundant amino acids (asp, glu, gly, val, phe, iso, leu, lys) in samples from the water column, sediments and sediment traps at 700 and 800 m depth. First, we calculated whether the composition of suspended matter samples (THAA, TN and amino acid mole-percentages) in near-bottom layers could be mimicked by mixtures of two end-members: (1) suspended matter from the water column (250 m depth) and (2) fine particles from the surface sediment (<63 μm). Then it was calculated whether mixtures of the same end-members could describe the composition of sediment trap samples from low flux periods (i.e. low resuspension). The average end-member mixture that best resembled both the suspended matter in the near-bottom layers as well as the low flux trap samples will be called 'fluff' *sensu* Bonnin et al. (2001) (Fig. 3.12a). The next step was to estimate whether the composition of the high flux trap samples (i.e. increased resuspension) could be described by mixtures of four end-members: (1) fluff, (2) fine particles (<63 μm) from the sediment top layer, (3) the 63-250 μm fraction from the sediment top layer and (4) coarse particles (>250 μm) from the sediment top layer (Fig. 3.12b). It was assumed that the composition of fluff was constant during periods of low and high flux. An extra constraint was taken into account to avoid multiple solutions: if the model calculated an addition of the >250 μm fraction, the 63-250 μm and the <63 μm fractions were also added according to their relative abundance in the sediment surface. Thus in case of resuspension of coarse particles at periods of high fluxes, we assumed that the sediment proper is resuspended in addition to the fluff. In the model the difference between the calculated THAA and TN concentrations and amino acid mole-percentages from the end-member mixtures and the measured values in the samples were minimized by using the Excel solver routine (least-squares).

At 700 m depth, a fluff mixture of 26±5% suspended matter from the water column and 74±5% of particles <63 μm from the sediment surface best described the near-

bottom suspended matter and low flux trap samples (Fig. 3.12a). According to the model, the high flux samples could be mimicked by 28% fluff with an addition of 72% sediment proper (consisting of additional fine particles: 2% <63 μm, but mostly of coarse sediment: 63% 63-250 μm and 6% >250 μm) (Fig. 3.12b). At 800 m depth, a fluff mixture of 16±19% suspended matter from the water column and 84±19% of fine particles from the sediment surface best described both the near-bottom suspended matter and low flux trap samples. The high flux trap samples at this depth were estimated being composed of 40% fluff with an addition of 60% sediment proper (consisting mainly of coarse sediment: 2% <63μm, 54% 63-250 μm and 4% μm). The estimated contributions of fluff to high flux trap samples at 700 and 800 agree well with the values reported by Bonnin et al. (2001) based on TOC (28-40% vs. ~40%).

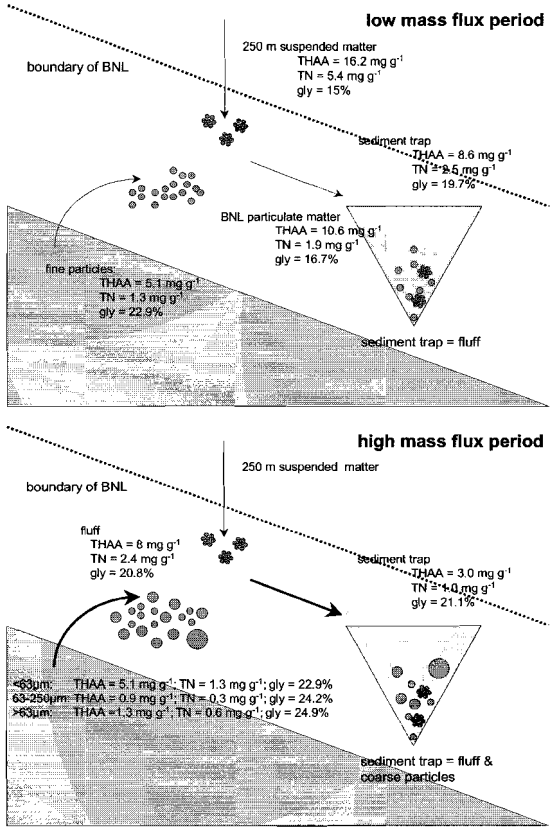

Figure 3.12 An end-member model based on TN, THAA and amino acid distributions was used to estimate the composition of near-bottom suspended OM, called fluff *sensu* Bonnin et al. (2001), from settling aggregates and resuspended particles from the sediment surface. Here, only mole-percentages of glycine were showed. In the model mole-% of the most abundant amino acids were used (asp, glu, gly, val, phe, iso, leu, lys). During **low flux** periods the fluff at 750 m depth was estimated as 74% of fine particles and 26% of suspended matter (250 m). OM in sediment traps during **high flux** periods (i.e. increased resuspension) was modeled as a mixture of 28% fluff, 2% of additional <63 μm, 63% of 63-250 μm and 6% >250 μm particles from the sediment.

The model results indicate that settling OM from the water column contributed only 16-26% to OM in the near-bottom layers during periods of low mass flux (low resuspension) and even less during periods of increased resuspension. Furthermore, fine particles appeared to be permanently present in the BNL. The addition of coarser particles, particularly the 63-250 μm fraction, seemed to cause the lower THAA concentrations and changing amino acid distributions during the period of high mass flux.

Studies of amino acids in sediment trap material (Lee, 1988; Haake et al., 1992; Haake et al., 1993; Nguyen and Harvey, 1997) and sediments (Henrichs et al., 1984; Sugai and Henrichs, 1992; Cowie and Hedges, 1992b; Boski et al., 1998; Dauwe and Middelburg, 1998; Grutters et al., 2001a) have indicated that the relative contribution of individual amino acids to THAA changes during OM mineralisation. From these relative changes in amino acid distribution a 'degradation index' was derived by application of a Principal Component Analysis (PCA) (Dauwe and Middelburg, 1998; Dauwe et al., 1999). Grutters et al. (2001a) established from application of a PCA to size fractionated sediments that the organic matter degradability decreased with water depth across the Goban Spur continental slope, which they explained by the continuous mineralisation of OM attached to fine particles during lateral transport. Here, we applied a PCA to data from suspended matter, sediment traps and sediments to examine whether the organic matter degradability decreases with depth across the Faeroe-Shetland slope. First, data from labile organic matter end-members (bacteria, phytoplankton and zooplankton) (Brown, 1991; Cowie and Hedges, 1992b; Dauwe and Middelburg, 1998) as well as data from refractory organic matter (Grutters et al., 2001a) were included in the PCA to determine the OM degradability in FSC samples relative to these OM end-members. A second PCA was applied to data from PROCS only to further identify changes in OM degradability across the FSC slope.

Here, classes of amino acids were used in the PCA instead of individual amino acids. BASIC amino acids (lysine, histidine, arginine) adsorb relatively easily to available mineral surfaces (Hedges and Hare, 1987; Henrichs and Sugai, 1993; Wang and Lee, 1993), and are therefore taken together. β-Alanine and γ-aminobutyric acid are referred to as NON PROTEIN amino acids since they are present in marine organisms in small quantities only and their relative amount increases with ageing of OM as a result of the decarboxylation of their precursor acidic amino acids (Lee and Cronin, 1982). The relatively stable glycine, serine and threonine are referred to as CELL WALL components (Burdige and Martens, 1988; Cowie and Hedges, 1992b; Cowie et al., 1992c). The relatively labile amino acids glutamic acid, tyrosine and phenylalanine (Hecky et al., 1973) are referred to as CELL PLASMA components. Methionine, valine, isoleucine and leucine have often been found to decrease during early diagenesis (Cowie and Hedges, 1992b; Cowie et al., 1992c; Dauwe and Middelburg, 1998) and are referred to as ESSENTIAL amino acids. Aspartic acid and alanine are referred to as ASP and ALA.

In the PCA including the organic matter end-members, 50% of the total variance in the samples was explained by the first Principal Component (PC), the second PC explained another 20%. The first PC was interpreted as an index for diagenetic degradation and correlated well with the ESSENTIAL amino acids (r^2=0.79; Fig. 3.13). The lowest degradation index was calculated for the phytoplankton, zooplankton and bacteria. All PROCS samples had an intermediate OM degradability relative to the labile and refractory organic matter end-members. The suspended matter samples were relatively fresh (high

organic matter degradability). The surface sediments had a lower organic matter degradability than the suspended matter, with the highest degradability at station 71 (750 m) and then decreasing to station 11 (370 m) > 61 (550 m) > 128 (900 m) > 54 (670 m) > 68 (850 m) > 78 (300 m). Differences between sediments and sediment traps were small, which can be explained by the presence of resuspended particles from the sediment surface in the traps.

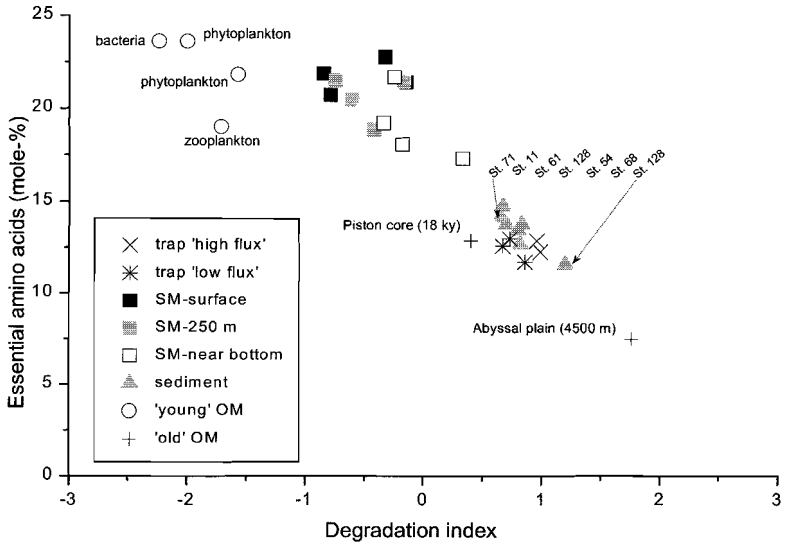

Figure 3.13 Results from a PCA applied to normalized data of suspended matter (SM) from the water column, sediment traps, and surface sediments. The PCA includes data from labile OM end-members as bacteria, phytoplankton and zooplankton (Brown, 1991; Cowie and Hedges, 1992b; Dauwe and Middelburg, 1998) and refractory OM samples (Grutters et al, 2001a). The variance along the 1st PC explained 50% of the total variance among the samples and was interpreted as a degradation index. The degradation index correlated well (r^2=0.79) with ESSENTIAL amino acids.

A second PCA was applied to PROCS samples only. The first PC explained 53% of total variance in the samples; the second PC explained another 19%. Variance along the first PC was determined by CELL PLASMA and ESSENTIAL amino acids, and to a slightly lesser degree by NON-PROTEIN and CELL WALL amino acids (Fig. 3.14). As in the first PCA, the position of the samples along the first PC can be interpreted as an indicator for its OM degradability, with fresh OM on the negative site of the first PC. Thus, the enrichment in cell plasma and essential amino acids in suspended matter samples from the water column confirms that OM in these samples is relatively fresh. OM degradability in the sediment trap samples was intermediate to that in the water column and the surface sediment samples, but mostly resembled the sedimentary OM degradability as was already demonstrated in Fig. 3.13. The NON-PROTEIN contents in the sediment trap samples and the sediments were similar, but were much higher than those in the suspended matter samples, indicating that organic matter in the sediments and traps has been subject to microbial degradation (Whelan, 1977; Lee and Cronin, 1982). The sediment trap samples (C5, C6) and sediments of the shallow stations

(78 and 11) as well as suspended matter in the water column at station 78 appeared to correlate to ASP. Sediments at stations 78 and 11 contain the highest amounts of calcium carbonate (respectively 47%, 39%; Fig 3.8c). Müller and Suess (1977) and Carter and Mitterer (1978) explained enrichments of aspartic acid by an association with calcium carbonate. Settling of these ASP-rich carbonate particles at the upper slope with subsequent erosion and down slope transport probably explains the enrichment of ASP in sediment trap samples.

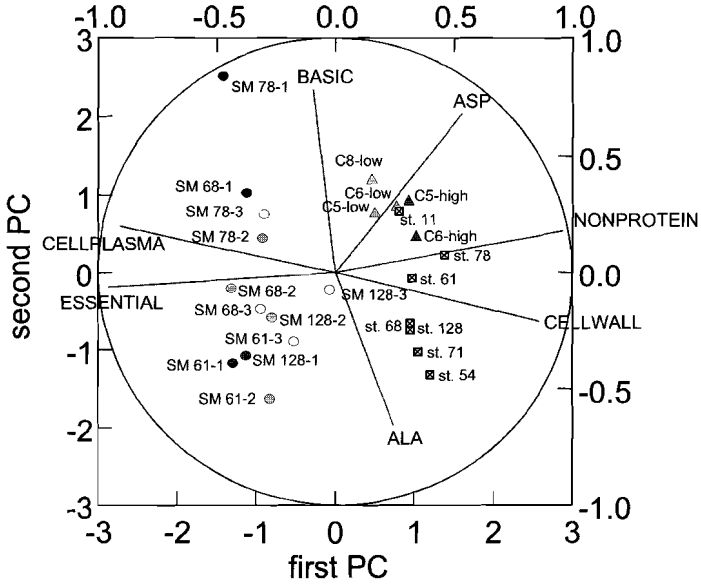

Figure 3.14 Results from a PCA applied to data from suspended matter, sediment traps and bulk sediments. The symbols ●, ◍ and ○ denote suspended matter (SM) samples from respectively the euphotic zone (no 1), 250 m depth (no 2) and near-bottom layers (no 3) at stations 78 (300 m), 61 (550 m), 68 (850 m) and 128 (900 m). The symbols ▲ and ▵ denote respectively high and low flux trap samples from moorings C5, C6 and C8. The □ denote bulk surface sediment from stations 78, 11 (370 m), 61, 54 (670 m), 71 (750 m), 68 and 128. Coefficients for standardised factor scores on the 1st and 2nd PC are given for classes of amino acids. BASIC denotes lysine, arginine, and histidine. NON-PROTEIN denotes β-alanine and γ-aminobutyric acid. CELL WALL denotes glycine, serine and threonine. CELL PLASMA denotes glutamic acid, tyrosine and phenylalanine. ESSNTIAL denotes methionine, valine, isoleucine and leucine. ALA denotes alanine, and ASP denotes aspartic acid.

The amount of particles <63 μm in the sediment surface increased from 8% at station 11 to 24% at station 71 (Fig 3.7), which confirmed earlier conclusions of Van Raaphorst et al. (2001) that sediment is preferentially deposited below 750 m depth. Moreover, ^{234}Th inventories (Fig. 3.8a) calculated in the upper 1 cm of the sediment sharply increased below 750 m depth. The input of ^{234}Th is assumed to be associated with the deposition of fine particles (Smith et al., 1993) at short time scales (half-live of ^{234}Th 24 days). The peak in the ^{234}Th inventory at 670-750 m depth as well as the maximum %AA-N of >70% at this depth (Fig 3.8f) demonstrates the deposition of young, amino acid-rich OM at this depth.

The relation between %AA-N and the mole-percentage of non-protein amino acids gives a more detailed view on the transport and degradation of amino acids across the slope

(Fig. 3.15). With increasing age of the organic matter, and hence its increasing microbial degradation and alteration, the %AA-N is expected to decrease whereas the mole percentage of the non-proteins would increase. Indeed most suspended matter samples have a low relative contribution of non-protein amino acids. The %AA-N in near-bottom suspended matter samples, however, was much higher. Thus suggests that particulate matter from the surface was likely not the dominant source of OM to the deeper water column. The %AA-N in near-bottom suspended matter decreased from the shallow slope (300 m) to the deeper slope (850-900 m) (Fig. 3.15). From 850 m to 900 m the relative contribution of non-proteins increased. The %AA-N and non protein composition of the surface sediment at the shallow slope (370 m) clearly differed from near-bottom suspended matter at 300 m, suggesting that near-bottom suspended matter did not settle at the shallow stations but was transported down slope. The OM in the sediment size fractions was more refractory than that in the water column considering the lower %AA-N and higher non-protein amino acid contributions, confirming the conclusions from the PCA. The finest fractions, however, appeared more labile than the coarser fractions and this was most pronounced at the deposition stations 54 (670 m) and 71 (750 m). The resemblance between the composition of particles <63 µm at these stations and the low and high flux sediment trap samples suggests that particularly fine particles from the surface sediment settled in the sediment traps during both periods, confirming the results of our end-member model.

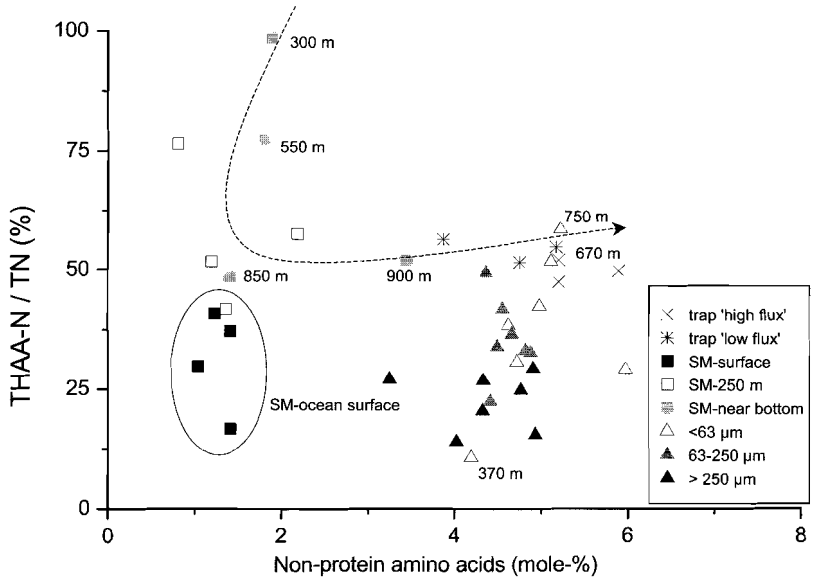

Figure 3.15 The relation between (1) the contribution of amino acid-N to TN (%AA-N) and (2) the mole percetage of non protein amino acids. The relation is shown for the suspended matter (SM) samples (■), high and low flux trap sampls (×) and sediment top layer (0-2.5 mm) size fractions (▲). The arrow indicates the route of SM transport in the near-bottom layer going from high %AA-N and low non-proteins at the upper slope to lower %AA-N and increased mole-% of non-protein amino acids at the lower slope. The shaded oval shows that OM in SM samples from the euphotic layer is very different from OM in the near-bottom layers, suggesting that they have different sources.

Conclusions

1. Across the slope of the FSC settling OM from the upper water column contributed little to the fluxes of OM in near-bottom sediment traps (16-26%).
2. During periods of low resuspension, the near-bottom water column mainly contained fine particles of <63 μm (~80%) from the surface sediments, indicating that these fine particles are 'semi-permanently' in suspension. During high flux events coarse material from the sediment surface was eroded.
3. The increasing mole-percentage of non-protein amino acids in near-bottom suspended matter point at ageing of the organic matter during down slope transport and further with depth in the sediment. However, fine particles containing young, highly reactive OM were eroded from the upper slope and preferentially settled at 750 m depth.

4.

Preservation of amino acids from in situ produced bacterial cell wall remnant peptidoglycans in marine sediments.

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Abstract

The enigmatic presence of intrinsically labile amino acids (Cowie and Hedges, 1992; Harvey et al. 1983; Lee, 1988) in old marine sediments (Cowie et al. 1995; Kawahata, 1993) is often explained by sorptive protection against enzymatic degradation (Keil et al. 1994b). Here, we show that a significant portion of the organic matter in marine sediments can be derived from bacterial cell walls enriched in D-amino acids. The analyses of amino acid enantiomers in marine sediments show the D/L ratio to increase with depth in the sediment mixed layer. Application of a transport-racemization-degradation model excludes a significant production of D-amino acids by racemization and implies *in situ* bacterial production as the main source. Amino acids associated with bacterial cell walls could account for approximately one third of the amino acids deeper in the sediments. We propose that in situ bacterial production and the primary flux of labile organic matter from the water column result in a small but highly reactive pool of amino acids in the surface mixed sediment only, whereas amino acids associated with refractory cell walls persist in marine sediments.

The contribution of amino acid-nitrogen to total nitrogen (%AA-N) as well as the spectrum of individual amino acids have been used as indicators of organic matter (OM) reactivity in marine sediments (Cowie and Hedges, 1992; Dauwe et al., 1999; Grutters et al., 2001a). Preferential enzymatic degradation of amino acids relative to bulk organic matter (OM) (Harvey, et al. 1983) causes the contribution of %AA-N to decline from 75-90% in fresh plankton to 40-50% in OM in sinking aggregates and further to 10-30% in sedimentary OM (Lee, 1988). However, the sorption of amino acids to mineral surfaces (Hedges and Hare, 1987; Henrichs and Sugai, 1993; Wang and Lee, 1993; Mayer, 1994a) and hence a lower susceptibility to enzymatic degradation (Schuster et al., 1998) is assumed to result in the preservation of these intrinsically labile compounds (Keil et al., 1994b). Another mechanism of amino acid preservation is perhaps the incorporation in peptidoglycans, the main structural components of bacterial cell walls (Schleifer and Kandler, 1972). It has been reported that peptidoglycans contribute significantly to dissolved organic matter in the deep ocean (McCarthy et al., 1998). Since bacterial cell walls are relatively resistant to enzymatic degradation (Schuster et al., 1998) we hypothesize that the in situ production of peptidoglycan contributes to the preservation of amino acids in marine sediments. In

addition, benthic production of peptidoglycan may be a source of suspended particulate organic matter in the lower water column of the ocean (Bauer and Druffel, 1998).

Particulate matter was collected from sediment traps (Antia et al., 1991), situated 400 meters above bottom (mab), at water depths of 1445 m and 3650 m across the Goban Spur N.E. Atlantic continental slope (49°24.89'N, 11°31.42'W – 49°05.30'N, 13°26.18'W). Sediment samples were taken from multicores and piston cores (representing ages of 7, 10, 18 ky) at water depths of 651 m, 1296 m, and 3650 m (Lohse et al., 1998). Sediment trap samples (pooled averages from individual cups covering a time span of 14 months) as well as sediment samples were analyzed for total hydrolysable amino acids (THAA) and D/L enantiomers (**methods**) of aspartic acid, glutamic acid, serine, and alanine, the major peptidoglycan amino acids. These amino acids were identified in all of the samples analyzed (Fig. 4.1). THAA concentrations decreased from 11-33 mg g^{-1} in the sediment trap samples to ~1-2 mg g^{-1} in the upper sediment layer (0-0.5cm). Concentrations further decreased from the sediment surface to the deeper layers with a strong gradient in the upper centimeters of the sediment, indicating the degradation of amino acids during early diagenesis (Grutters et al., 2001a) (Fig. 4.2). The concentration of D-amino acids (Fig. 4.3) increased in the upper centimeter of the sediment (glutamic acid, serine) or was constant with sediment depth (aspartic acid, alanine), whereas the concentration profiles of L-amino acids closely followed THAA concentration profiles. Concentrations of both D- and L- amino acids in the piston core samples were lower than in the sediment mixed layer. This could indicate the degradation of both enantiomers (O'Dowd, 1998) deeper in the sediment or their incorporation into refractory geopolymers (Knicker, 2001). D/L amino acid ratios were low in sediment traps as well as in the upper sediment layer and increased strongly within the sediment mixed layer (Fig. 4.4). Apart from glutamic acid, D/L ratios in the piston cores fitted with those in the sediment mixed layer.

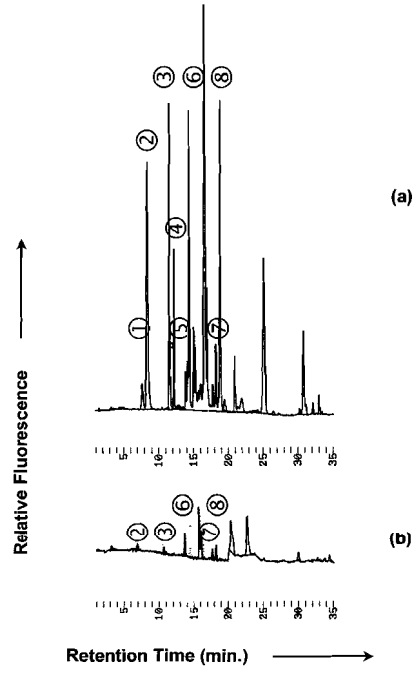

Figure 4.1 The 5 to 30 min region of the reverse-phase HPLC chromatograms of a 6 M HCl hydrolyzed desalted sediment surface sample (station PE95-6, 1296 m) (a). Also shown is a chromatogram of a procedural blank (b). Peaks were identified by comparison of the retention times of an amino acid standard run at the same time. Peak identifications of the amino acids discussed in the text: (1) D-aspartic acid; (2) L-aspartic acid; (3) L-glutamic acid; (4) D-glutamic acid; (5) D-serine, (6) L-serine; (7) D-alanine; (8) L-alanine.

The potential contribution of D-amino acids by racemization, a process by which L-amino acids are converted into D-amino acids, was evaluated by application of a two-layer (0-8cm, >8cm) diagenetic model (**methods**), including sedimentation (Van Weering et al., 1998), sediment mixing, degradation (Grutters et al., 2001a), and racemization (Bada and Schroeder, 1975). In both layers a labile and a refractory fraction was taken into account (Fig. 4.2). The racemization rates used were derived from values reported for free amino acids (Bada and Schroeder, 1975). The initial concentrations of L-amino acids at the sediment-water interface were calculated from measured concentrations in the sediment extrapolated to the sediment surface. The concentration of D-amino acids at the sediment water interface was set to zero. This was supported by the low D/L ratios in the sediment traps (apart from aspartic acid), which indicated that settling organic matter is poor in D-amino acids. The model output from the lower boundary of the sediment mixed layer was used as input for the deeper zone. According to our model, the rapid increase in measured D/L ratios with sediment depth cannot be explained with racemization alone, pointing at an additional source of D-amino acids in the sediment mixed layer.

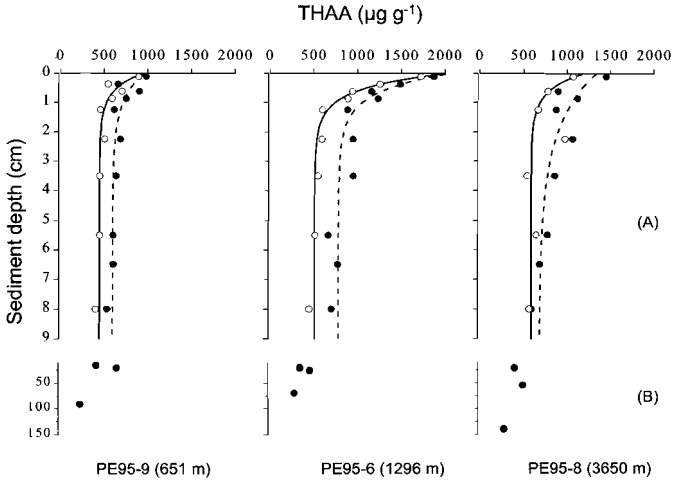

Figure 4.2 THAA concentration profiles (mg g^{-1}) (●) and profiles corrected for the amount of cell wall-associated amino acids (AA_{CW})(○) in the sediment mixed layer (A), and piston cores (B) at 651 m, 1296 m, and 3650 m water depth across the Goban Spur continental slope. Piston core samples represented ages of 7, 10, and 18 ky, determined from foraminiferal stratigraphy. The area between the solid and dashed line denotes the contribution of AA_{CW} to THAA. THAA concentration profiles and the remaining profiles after correction in the sediment mixed layer were fitted by application of a 'diffusion-reaction' model.

The calculated contribution of D-amino acids by racemization based on our model was subtracted from the measured concentrations. The remaining concentration of D-amino acids is considered to represent the contribution of D-amino acids from bacterial peptidoglycan, derived from either living bacteria or cell wall remnants. If we assume that the observed D-amino acid profiles (Fig. 4.3) reflect living bacteria only, published D/L ratios from bacterial cultures (Pedersen et al., 1999) (D/L_{LB}) can be used to estimate the contribution of amino acids from living bacteria (AA_{LB}) to the measured THAA as $\Sigma(D^*(1+(D/L)_{LB}^{-1}))$. We take into account that Gram-negatives comprise 90% of the total

bacterial community in aerobic surface sediments and ~70% in anaerobic, deeper sediment layers (Moriarty, 1982). In this case, the total AA_{LB} would correspond to 11-57% of the measured THAA in the sediment trap samples and in the upper sediment layer but would exceed the measured THAA concentration up to a factor of 5 in the sediment mixed layer. Furthermore, the bacterial abundance as estimated from calculated AA_{LB}, using empirical relationships between amino acid-nitrogen, bacterial cell-nitrogen and cell abundance (Simon and Azam, 1989), would exceed reported values of cell abundance for marine sediments (Deming, 1985; Craven, 1986; Schmidt et al. 1998; Danovaro et al., 1998; Boetius et al., 2000) up to a factor of 1500. From these calculations it is concluded that deeper in the sediment mixed layer living bacteria cannot exclusively explain the observed profiles of D-amino acids. Thus, a significant contribution of D-amino acids has to be inferred from bacterial cell wall remnants.

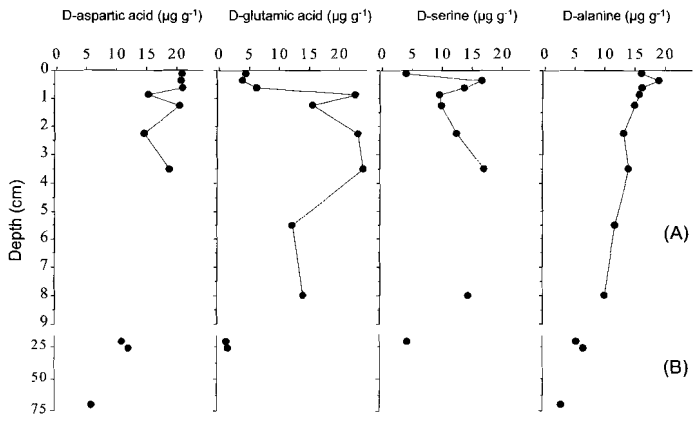

Figure 4.3 Profiles of D-amino acids (µg g^{-1}) in the sediment mixed layer (A) and the piston core samples (B) from station PE95-6 (1296 m).

The contribution of cell wall-associated D- and L-amino acids (AA_{CW}) to THAA in the sediment mixed layer were calculated using D/L ratios from peptidoglycan (D/L_{PEP}) as $1.2*\Sigma(D*(1+(D/L)_{PEP}^{-1}))$. D/L_{PEP} ratios were estimated from those types of peptidoglycan known to contain D-and L-aspartic acid, glutamic acid, serine, and alanine (Schleifer and Kandler, 1972). The factor 1.2 corrects for the amino acids in peptidoglycans other than these four D- and L-amino acids (Schleifer and Kandler, 1972). The contribution of AA_{CW} to THAA was only 1-4% in the sediment trap samples, and ranged between ~10-43% deeper in the sediment mixed layer (Fig. 4.2). Since our D/L values are on the low side relative to recently reported values (Amon et al., 2001) for bacteria, cyanobacteria and DOM these are conservative estimates only.

The estimated AA_{CW} were subtracted from the measured THAA concentrations to determine the pool of labile THAA. Profiles of labile THAA show a steeper concentration gradient in the upper layers of the sediment than those of uncorrected THAA (Fig. 4.2).

First-order degradation rate constants of labile THAA, obtained by fitting THAA profiles with a diagenetic model (**methods**) (Fig. 4.2), ranged between 1.4 and 8.4 y^{-1}, exceeded values from uncorrected profiles (Grutters et al., 2001a) by a factor of 2-10, and are consistent with amino acid degradation constants determined from laboratory experiments on amino acid degradation (Harvey et al., 1983; Dauwe et al., 1999). This small but reactive pool of amino acids in the upper sediment layers may originate from settling organic matter and in situ production of cell plasma. We propose that the concomitant production of refractory bacterial cell walls, explaining at least one third of the amino acids in the sediment mixed layer, may be the first step in the preservation of amino acids and hence initiates preservation of organic matter.

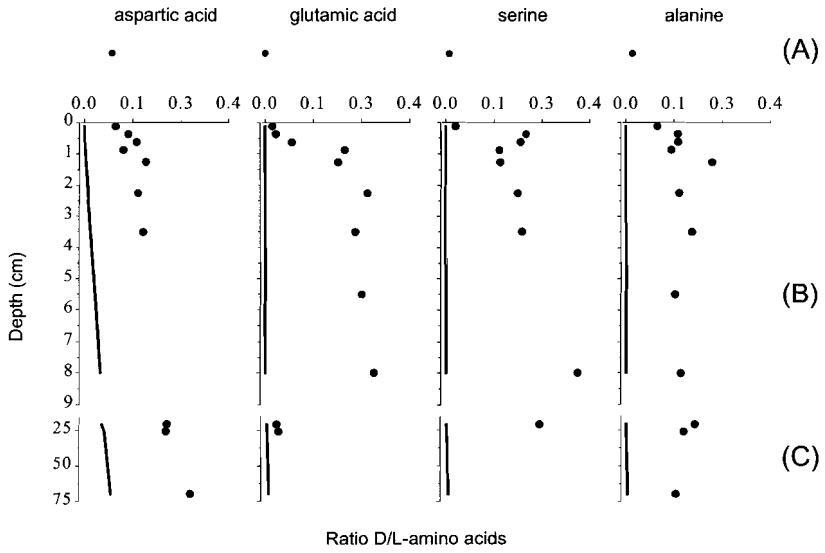

Figure 4.4 D/L ratios of the enantiomers of aspartic acid, glutamic acid, serine and alanine in samples from sediment traps (A), the sediment mixed layer (B), and piston cores (C) from station PE95-6 (1296 m). Solid lines show D/L ratios calculated with the 'sedimentation-mixing-degradation-racemization' model (**methods**).

Methods

Total hydrolysable amino acids (THAA) and amino acid enantiomers

THAA were measured by reverse-phase high performance liquid chromatography (HPLC) analysis, after liquid phase hydrolysis (110°C for 24 h) and pre-column derivatization with *o*-phtaldialdehyde (OPA) (Grutters et al., 2001a). Amino acid enantiomers were analyzed by HPLC after vapor phase hydrolysis at 150°C for 3 hours and pre-column derivatization with *o*-phtaldialdehyde/*N*-acetyl-L-cysteine (OPA/NAC) and UV-fluorescence detection (Glavin et al., 1999). Prior to derivatization hydrolysates were desalted by using cation exchange resin (AG50W-X8, Bio-Rad) and the desalted extracts were stored in borate buffer (pH 9.4). Concentrations of the THAA and enantiomers were calculated by comparison of the amino acid peak areas to those of a standard run in parallel. Very low levels of amino acids were detected in a blank carried through the same processing

procedure as the sediment samples (Fig. 4.1) and were subtracted from the measured peak areas of the samples. Precision, expressed as the coefficient of variation (CV), for duplicate samples varied between 5-15% for all amino acids except tyrosine (33%). The CV of amino acid enantiomers, determined from duplicate samples, was 3% for aspartic acid, 40% for glutamic acid, 21% for serine and 15% for alanine. The reproducibility of the enantiomer analysis, determined from triplicate injections of several individual samples, was 1% for aspartic acid and alanine, 5% for serine and 15% for glutamic acid.

'Racemization' model

The equations for racemization of D- and L-amino acids (Bada and Schroeder, 1975) were extended with sedimentation (Van Weering et al., 1998), sediment mixing and degradation (Grutters et al., 2001a). The general solution for both enantiomers becomes

(Eq. 1) $L(x) = 1/2(L_0 - D_0)e^{Ax} + 1/2(L_0 + D_0)e^{Bx}$

(Eq. 2) $D(x) = L - (L_0 - D_0)e^{Ax}$

where the coefficients A and B stand for:

$$A = \frac{\omega - \sqrt{(\omega^2 + (8r + 4m)D_b)}}{2D_b}; \qquad B = \frac{\omega - \sqrt{(\omega^2 + 4mD_b)}}{2D_b}.$$

Coefficients r, m, ω, D_b, z represent the first-order racemization rate constant (y^{-1}), first-order degradation rate constant (y^{-1}), sedimentation rate (cm y^{-1}), sediment mixing coefficient (cm^2 y^{-1}), and sediment depth (cm, positive downwards) respectively. Racemization rates were estimated by interpolating rates reported for free amino acids at 0°C and 25°C (Bada and Schroeder, 1975) to the in situ temperature of 4°C using the Arrhenius equation. Equal rates for racemization as well as degradation were assumed for D- and L-enantiomers. It was taken into account that aspartic acid does not linearly racemize but rather has a 'fast' and a 'slow' component (Goodfriend, 1991; Collins et al., 1999). An aspartic acid racemization rate was derived from running a model (Collins et al., 1999) under boundary conditions that mimic the sedimentary environment at the Goban Spur continental slope. The distribution of a labile and non-degradable fraction was determined from THAA concentration profiles (Fig. 4.2) as ($THAA_0$-$THAA_\infty$) / $THAA_0$ * 100. Below the sediment mixed layer, the concentration of enantiomers was calculated as a function of first-order degradation and racemization only, according to $L=0.5(L_1-D_1)exp(-2rt-mt) + 0.5(L_1+D_1)\ exp(-mt)$ and $D=L-(L_1-D_1)exp(-2rt-mt)$. For t we used the age at the lower boundary of the sediment mixed layer (~3,000 y) to 18000 y, the age of the oldest piston core samples. L_1 and D_1 refer to concentrations of L- and D-enantiomers at the boundary at the sediment mixed layer calculated from Eqs. 1 and 2.

'Reaction-diffusion' model

THAA profiles were fitted with a 'diffusion-reaction' model according to $C_{THAA}(z)=C_{L,0}exp(-z\sqrt{(k/D_b)})+C_{ND}$ in which C_{THAA} is the concentration of amino acids in the sediment. $C_{L,0}$ and C_{ND} are the labile and non-degradable fraction of the THAA (mg g^{-1}) at the sediment surface; z is the sediment depth (cm) positive down core; D_b is the sediment mixing coefficient (cm^2 y^{-1}) derived from modeling TOC concentration profiles (Grutters et al., 2001a), and k is the first-order degradation constant of THAA (y^{-1}). $C_{L,0}$, C_{ND} and k were estimated by fitting the concentration profiles.

5.

Mid-slope accumulation of D-amino acids associated with bacterial cell walls across the Faeroe-Shetland Channel (North Atlantic Ocean).

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Abstract

In the framework of the PROCS project (PROcesses on the Continental Slope), we present data on DL-amino acid enantiomers in samples from suspended matter, near-bottom sediment traps, and surface sediments across the SE slope of the Faeroe-Shetland Channel (FSC). The aim of this study was to test the hypothesis that part of the mid-slope accumulation of D-amino acids is associated with bacterial cell walls that were produced in near-bottom water layers. The ratio of D over L-amino acids increased from the euphotic water layer to the sediment surface and further in the deeper sediments. An end-member model was developed based on D-amino acids in suspended matter, sediment traps and surface sediments. The model demonstrated that the distribution of amino acid enantiomers across the FSC is correlated to the distribution of total hydrolysable amino acids (THAA), and that D-amino acids in the bottom water were not derived from the euphotic zone but from fine particles resuspended from the sediment surface. On average, approximately 5% of the D-amino acid concentration in the sediments could be accounted for by a-biotic racemization. Thus, the D-amino acids appeared to be associated with peptidoglycan in bacterial cell wall remnants, and their contribution to THAA increased to ~24% in the upper 5 cm of the sediment. It is concluded that that bacterial growth on fine, labile organic matter-rich particles transported down-slope in near-bottom water layers results in a mid-slope accumulation of refractory bacterial cell wall material, thereby enhancing the preservation of amino acids in the sediment at this depth.

Introduction

Lateral transport of sediment particles across the continental slope is an important source of organic matter (OM) deposition to deep-sea sediments (Biscaye and Anderson, 1994; Antia et al., 1999; Epping et al., 2001), in addition to vertical settling by aggregates from the euphotic zone (Honjo et al., 1982a; Fowler and Knauer, 1986). At the upper continental slope nepheloid layers may be generated due to strong bottom currents (McCave, 1986) and by internal wave activity (Dickson and McCave, 1986), depending on sediment bed structure (Thomsen, 1999). During transport in the BNL repetitive cycles of resuspension-deposition and disaggregation-aggregation hydrodynamically sort small from coarse particles (Thomsen, 1999). Therefore, the down slope accumulation of fine particles that are relatively rich in OM due to their large specific surface areas (Mayer, 1994a; 1994b) could explain the elevated OM concentrations in the sediments with increasing water depth across the NE Atlantic slope (Grutters et al., 2001a; Epping et al., 2001).

During the down slope transport of organic matter-rich aggregates bacterial activity is stimulated (Turley and Lochte, 1990; Ritzrau, 1996; Boetius et al., 2000a; Nagata et al., 2000), and the labile compounds such as sugars and amino acids are rapidly degraded with rates of 20-35 y^{-1} (Westrich and Berner, 1984; Harvey et al., 1995). Epping et al. (2001) demonstrated that the age of OM at the lower slope of the Iberian margin is approximately 550 days. Thus, down slope particle transport increases the time OM spends in the water column by an order of magnitude relative to vertical settling, thereby extending the degradation before ultimate deposition. Hence, at continental margins where lateral, down-slope transport dominates over vertical settling the OM deposited at the sediments becomes increasingly aged and refractory with increasing depth across the slope. Earlier reported results on total nitrogen (TN) and total hydrolysable amino acids (THAA) concentrations in suspended matter, sediment traps and surface sediments across the slope of the Faeroe-Shetland Channel showed, however, that fine, amino acid-rich, particles accumulated in the surface sediment at ~750 m depth (Grutters, 2001c). It was concluded that these amino acids were most likely derived from secondary production in near-bottom layers and the sediment surface, and not from primary production in the euphotic zone of the water column.

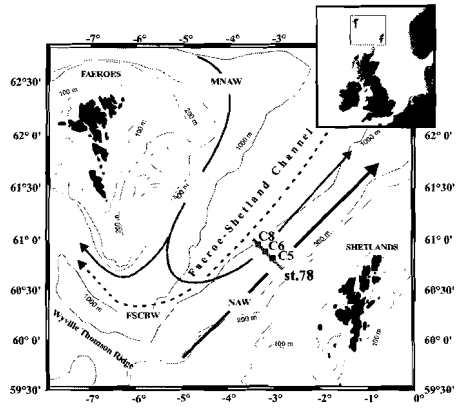

Figure 5.1 Location of sampling stations across the slope of the Faeroe-Shetland Channel. Sediment trap samples were obtained from moorings C5 (700 m), C6 (800 m), and C8 (1000 m) (squares). The solid line through the mooring sites represents the transect where suspended matter and sediment samples were collected. Sediment samples were obtained from station 78 (300 m), 11 (370 m), 61 (550 m), 54 (670 m), 71 (750 m), 68 (850 m) and 900 m). Suspended matter samples were taken at the position of station 78, 61, 68 and 128 from the euphotic layer of the ocean, 250 m depth and 10 meter above the bottom.

In this study, we present results on DL-amino acid enantiomers in the same samples as described in Grutters et al. (2001c). We will test the hypothesis that part of the mid-slope accumulation of D-amino acids is associated with bacterial cell walls that were produced in near-bottom water layers. D-amino acids are generally only produced from L-amino acids in proteins by a-biotic racemization on time scales of a few million years. Bacteria, however, are among the few organisms capable of synthesizing several D-amino acids as constituents of peptidoglycans, structural components of their cell walls (Meister, 1965;

Schleifer and Kandler, 1972). Several studies have demonstrated that D-amino acids are ubiquitous in the water column and sediments that are too young for any significant contribution by racemization (Lee and Bada, 1977; Bada and Lee, 1977; McCarthy et al., 1998; Pedersen et al., 1999; Grutters et al., 2001b), evidencing that amino acids from bacterial cell walls may importantly contribute to THAA in the ocean. Due to the relative resistance of bacterial cell walls against degradation (Rogers, 1983) they can accumulate in the sediments (Pedersen et al., 1999; Grutters et al., 2001b). From a simple end-member model, based on DL-alanine, DL-glutamic acid, DL-aspartic acid and DL-serine in suspended matter samples, sediment traps and surface sediments we will demonstrate that the source of D-amino acids across the FSC slope is correlated to that of THAA. Concentrations of D-amino acids (lowered for racemization as estimated from a diagenetic 'racemization-degradation' model) will be used to estimate the contribution of bacterially derived amino acids to THAA. Further, the relative importance of whole cells and/or cell wall remnants in the preservation of amino acids is discussed.

Material and methods

Study Area-The Faeroe-Shetland Channel (60°N, 6°W-63°N, 1°W) connects the Norwegian Sea with the Atlantic Ocean. It has a maximum depth of 1500-2000 m at the northeastern entrance and is about 600-650 m deep at the Wyville-Thomson Ridge in the south (Fig. 5.1). The upper 200-500 m of the water column is composed of two distinct water masses, North Atlantic Water (NAW) flowing northward along the West Shetland shelf and Modified North Atlantic Water (MNAW) flowing southward across the Faeroe Shelf and upper northwestern slope. This MNAW turns in front of the Wyville-Thomson ridge and then runs northward parallel to NAW along the central axis of the Channel. Deep water (>500 m) comes from the Norwegian Basin and flows southward towards the Wyville-Thomson Ridge and further. The morphology and hydrography of the margin are described in detail by Stoker et al. (1993) and Turrell et al. (1999).

Samples-Samples from the water column, sediment traps and surface sediments were collected along a transect across the SE slope of the Faeroe-Shetland Channel (60.73°N, 2.87°W; depth 300 m-61.00°N, 3.18°W; depth 1000 m) with R.V. *Pelagia* during the PROCS 99-1 cruise from 14 April-5 May 1999.

Suspended matter samples were collected at stations 78, 61, 68 and 128 (Fig. 5.1) with a CTD-Rosette system holding 22 12-l NOEX bottles. An amount of 40 liters was collected from the surface waters, 250 m water depth, and ~10 m above the bottom (mab). In a thermostated laboratory at ambient bottom water temperature the water was filtrated through precombusted Whatman GFF filters (0.7 μm). Filters were stored frozen (-20°C) until analysis at the NIOZ.

Modified Technicap PPS 4/3 cylindrical sediment traps (aspect ratio 8) were positioned 2 and 30 mab at moorings C5, C6 and C8 (Fig. 5.1) and sampled at 1-day intervals. Samples were stored at 4°C until further processing at the NIOZ. For a detailed description of the moorings as well as of the sample preparation and collection we refer to Bonnin et al. (2001). At the NIOZ, the sediment trap samples were filtrated over 0.4 μm polycarbonate filters (47 mm diameter), washed with 2 ml of MilliQ water and freeze-dried.

After freeze-drying the sediment material was carefully removed from the polycarbonate filters and stored in clean glass vials with PTFE-lined caps until analysis.

Sediment samples were collected with a multi-corer or box-corer at stations 78, 11, 61, 54, 71, 68 and 128 (Fig. 5.1) and processed in a thermostated laboratory at ambient bottom water temperature directly upon retrieval. Cores were sliced into 4 intervals of 2.5 mm (0-1 cm), 5 mm (1-3 cm), 10 mm (3-7 cm), and 20 mm (7-15 mm), using the high precision hydraulic core-slicer as described in Van der Zee et al. (2001). The sediment slices were centrifuged (3000 rpm, 10 min) for pore-water extrusion and freeze-dried. The freeze-dried samples were homogenized and ground by an automated tungsten mortar. Splits of the sediment surface samples were fractionated into three size fractions (<63 μm, 63-250 μm and >250μm) by dry sieving. The bulk sediment and fractionated samples were kept in clean glass vials with PTFE-lined caps until analysis.

Total organic carbon (TOC) and total nitrogen (TN)-Filtrated freeze dried filters from the suspended matter samples as well as freeze-dried, homogenized and ground samples from the sediment traps and sediment slices were analyzed for TOC and TN on a Carlo-Erba 1500 elemental analyzer. For TOC determination, carbonates were removed by addition of sulphurous acid, following the procedure of Verardo et al. (1990) and modified after Lohse et al. (1998, 2000).

Total hydrolysable amino acids- Sample preparation was described in detail by Grutters et al. (2001c). Briefly, an amount of 50 μl filtrated hydrolysate (hydrolysis of ~0.2 mg sediment for 24 h at 110°C) was evaporated and washed three times with 150 μl MilliQ water. The acid free residue was dissolved in 1000 μl sodium acetate buffer (pH 7). An aliquot of the dissolved residue was diluted with sodium acetate buffer (pH 7) until the concentration fell in the 150-1500 nM range. The samples were analyzed for amino acids by reverse-phase HPLC analysis and pre-column reaction with *o*-Phtaldialdehyde (OPA) (Lindroth and Mopper, 1979; Cowie and Hedges, 1992a). Amino acid concentrations were calculated from peak areas, which were calibrated with a standard amino acid mixture ranging in concentration from 150 to 1500 nM (r^2>0.95). Peak areas were corrected for procedural blanks. The reproducibility of the analysis, determined from triplicate injections, was better than 4% (expressed as CV) for all amino acids. The precision of duplicate samples was better than 19% for all amino acids.

Amino acid enantiomers-Samples were prepared similarly to those for the THAA analysis, and analyzed for amino acid enantiomers by reverse-phase HPLC and pre-column derivatized with *o*-phtaldialdehyde/*N*-isobutyrylcysteine as described by Fitznar et al. (1999). A ternary gradient was used to separate D- and L-amino acids, the mobile phase consisted of two 25 mM sodium acetate buffers (pH 7.0 and 5.3, respectively) and methanol. Enantiomers were measured fluorimetrically at an excitation wavelength at 330 nm and an emission wavelength at 445 nm. Concentrations of the amino acid enantiomers were calculated from peak areas, which were calibrated with a standard amino acid mixture ranging in concentration from 50 to 1000 nM. Here, only the results from DL-aspartic acid, DL-glutamic acid, DL-serine, and DL-alanine will be discussed. For these amino acids, the correlation (r^2) between peak areas and standard concentration was >0.99. The precision of

duplicate samples (expressed as CV) ranged between 16-21% for the D-amino acids, and ranged between 4-7% for the L-amino acids.

Results

Suspended matter in the water column

The concentrations of L-amino acids decreased when going down in the water column at the upper slope (station 78-300 m, station 61-550 m) (Fig. 5.2). At the lower slope (station 68-850 m, station 128-900 m) the concentration increased from the euphotic layer to 250 m but was lower again close to the bottom. The concentrations decreased in lateral, off shelf direction but increased again at the deepest part of the FSC at all three depths in the water column.

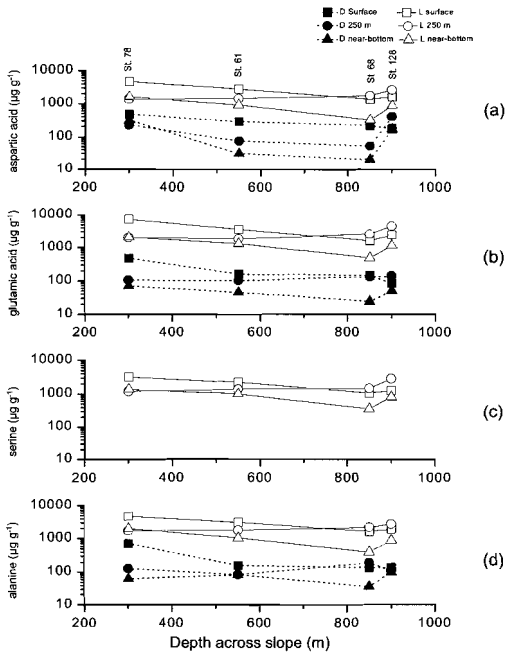

Figure 5.2 Concentrations of DL-aspartic acid (a), DL-glutamic acid (b), DL-serine (c) and DL-alanine (d) in suspended matter samples collected at stations 78 (300 m), 61 (550 m), 68 (850 m) and 128 (900 m). Suspended matter samples were taken in the euphotic surface layer (■), 250 m (●) and near the sea floor (~10 mab) (▲). The D-amino acids are shown with solid symbols, the L-amino acids are shown with open symbols. D-serine was below detection in the suspended matter samples.

The concentrations of D-amino acids were one order of magnitude lower than of L-amino acids, and generally decreased with depth in the water column at station 78, 61 and 68 (Fig. 5.2). At the lower slope (station 128) the concentrations increased from the euphotic layer to 250 m and then decreased again. The concentrations in the euphotic layer decreased in lateral direction from the upper slope towards the center of the FSC. Deeper in the water column (250 m and near-bottom) the concentrations first decreased off shelf but increased again in the deepest part of the FSC.

The ratio of D/L-aspartic acid (Fig. 5.3) increased with depth in the water column at the upper slope (300 m) and at the lower slope (900 m). At the stations in between (550 m and 850 m) the D/L-aspartic acid ratio decreased with increasing water depth. The D/L-

aspartic acid ratio in the surface layer did not change much going off shelf towards the center of the FSC. The D/L-aspartic acid ratio in the deeper water layers (250 m and near-bottom) first decreased off shelf and then increased again at the lower slope. For the D/L ratios of the other amino acids there was little difference with depth in the water column as well as in lateral direction from the upper slope towards the center of the FSC.

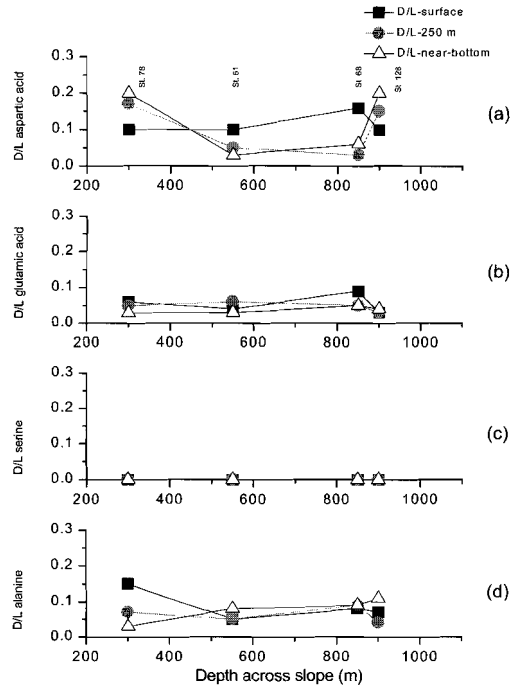

Figure 5.3 Ratios of D/L-aspartic acid (a), D/L-glutamic acid (b), D/L-serine (c) and D/L-alanine (d) in suspended matter samples collected at stations 78 (300 m), 61 (550 m), 68 (850 m) and 128 (900 m). Suspended matter samples were taken in the euphotic surface layer (■), 250 m (●) and near the sea floor (~10 mab) (▲). D-serine was below detection in the suspended matter samples.

Sediment traps

Total mass flux (TMF) as well as TOC and TN contents of the sediment trap samples from the moorings C5 (700 m), C6 (800 m) and C8 (1000 m) were described in detail by Bonnin et al. (2001). At C5 and C6 TMF sharply increased at yearday 112 (Fig. 5.4), and decreased again to its background value after yearday 115. At C8 the TMF was low during the whole trapping period. For C5 and C6, samples taken from day 112 to day 115 will be referred to as "high flux" samples. The samples from the remaining days as well as the samples from C8 will be referred to as "low flux" samples.

Here, we would like to focus on the differences between periods of "high" and "low" TMF, and therefore average L- and D-amino acid concentrations of the two sediment traps attached to each mooring (2 and 30 mab) are reported. The concentration of the four L-amino acids did not vary much between the traps and was lower during periods of "high" TMF than during periods of "low" TMF (Fig. 5.4 and 5.5). The concentrations of D-amino acids followed the same pattern as L-amino acids although the difference in concentration

between periods of "high" and "low" TMF was less pronounced for D-glutamic acid and D-serine (Fig. 5.5).

The ratio of D/L-aspartic acid (Fig. 5.5) in "high flux" samples is somewhat lower than in "low flux" samples, and both are similar to those of the suspended matter samples. By contrast, the D/L-ratio of the other amino acids was higher in "high flux" samples than in "low flux" samples. There was little difference in D/L-ratios with increasing depth across the slope (going from C5 to C8).

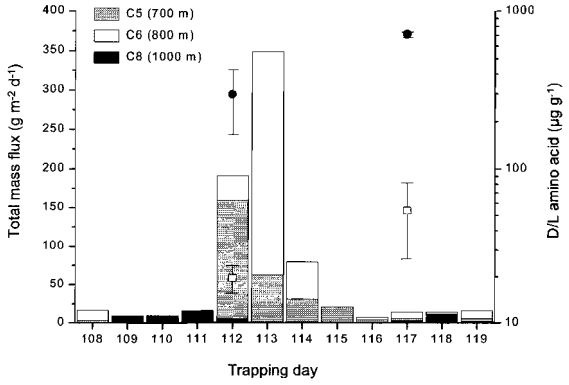

Figure 5.4 Total mass fluxes as measured in the near-bottom sediment traps (2 mab) attached to moorings C5 (700 m), C6 (800 m) and C8 (1000 m). For a more detailed description of mass fluxes in the sediment traps, we refer to Bonnin et al. (2001). The sampling period was 12 days. The period between yearday 112 and 115 is referred to as "high flux" period, and the remaining period (yearday 108-111, 116-119) is referred to as "low flux" period. Also shown are the D-aspartic acid (□) and L-aspartic acid (●) concentrations in a "high" and "low flux" sample from mooring C5. These DL-amino acid concentrations are average values of the two traps that were attached to the moorings (2 mab and 30 mab) and demonstrate that the concentrations were higher during "low flux" periods than during "high flux" periods.

Sediments

Visual inspection of the sediment cores showed that at stations 78 and 11 (upper slope) the sediment consisted of brown colored clays that were overlain by ~5 cm of grayish-brown coarse sand (Fig. 5.6). At station 71 (mid slope) clay was not observed in the upper 10 cm. At station 128 (lower slope) clay was observed only below 9 cm sediment depth. The transition from clay to coarse sands was interpreted as the transition from glacial to interglacial conditions (Stoker et al., 1991) and illustrates that since the early Holocene sediment accumulation was stronger at the lower than at the upper slope (maximum at 750 m depth). The visual observations of the sediment texture correspond with the median grain size distributions, obtained from analysis with a Coulter LS230 laser grain-sizer. The average median grain size for the upper ~3 cm of the sediment decreased from ~400 µm at the upper slope to 149 µm at the lower slope (Grutters et al., 2001c).

At stations 11 (370 m) and 128 (900 m) the concentration of L-amino acids decreased with sediment depth, with a sharp sub-surface peak at 2.5-5 mm (Fig. 5.6). At station 71 (750 m) the concentration was constant in the upper 3 cm and then decreased with sediment depth. The concentration of L-amino acids in the sediments of station 71 and 128 was almost twice as high as of station 11. The profiles of D-aspartic acid, D-glutamic

acid and D-alanine resembled those of the L-amino acids at all stations, albeit with a less clear decrease with sediment depth. The D-serine concentration was more or less constant with depth. Like the L-amino acids, the concentration of D-amino acids at station 71 and 128 was higher than at station 11, most clearly for D-glutamic acid and D-alanine.

The ratios of D/L-aspartic acid and D/L-alanine at station 11 increase from the sediment surface to ~5 cm depth and then decrease further down core. At the lower-slope stations 71 and 128 the D/L-aspartic acid and D/L-alanine ratio are lower than at station 11 and show a minor increase down-core only. The D/L-ratio of glutamic acid and serine in the sediments is similar at all stations, and slightly increase with depth in the sediment.

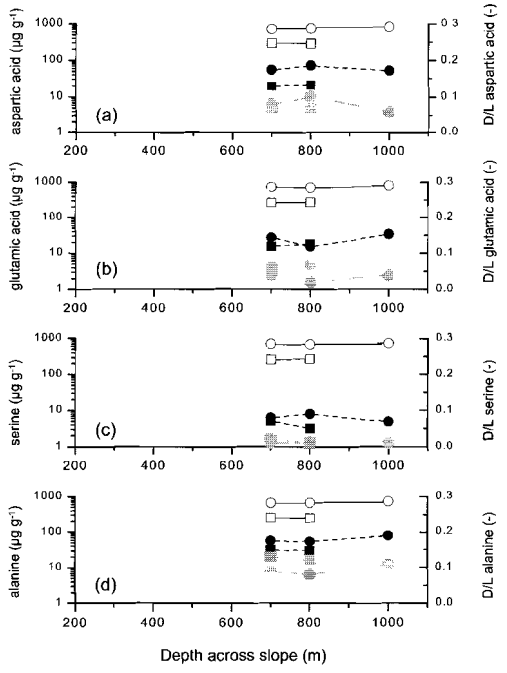

Figure 5.5 Concentrations (left axis) and D/L ratios (right axis) of aspartic acid (a), glutamic acid (b), serine (c) and alanine (d) in sediment trap samples collected from moorings C5 (700 m), C6 (800 m) and C8 (1000 m). D-amino acids are represented by solid symbols, L-amino acids by open symbols, and D/L-ratios by gray symbols. A distinction was made between "high flux" (□) and "low flux" samples (○). The concentrations of DL-amino acids in the traps are average values of the two sediment traps (2 mab and 30 mab) that were attached to each mooring.

Figure 5.7 shows the total concentration of the four L-and D-amino acids in the upper 0-2.5 mm of the sediment (both bulk sediment and size fractions). The concentration of L-amino acids increased with depth across the slope with maximum concentrations at station 71 (750 m). The concentration of L-amino acids in the coarse size fractions (63-250 and >250 µm), are similar to those in the bulk sediment. The concentration in the finest fraction also increases with depth across the slope with a pronounced maximum at station 71. The concentration of D-amino acids shows the same pattern, with a maximum concentration at station 71. The concentrations in the coarse size fractions are somewhat lower than in the bulk sediment, whereas the concentration in the finest fraction is considerably higher with a clear maximum at station 71.

The ratio of D/L-amino acids, obtained from the summed D-amino acids over the summed L-amino acids in the sediment top layer, was approximately constant across the slope with an elevated ratio at station 61 only (Fig. 5.7). The D/L-ratio in the 63-250 µm fraction was similar to that of the bulk sediment. The D/L-ratio in the finest fraction and in the coarse fraction was higher than in the bulk sediment at the upper slope, but lower than in the bulk sediment at the mid-slope and lower-slope.

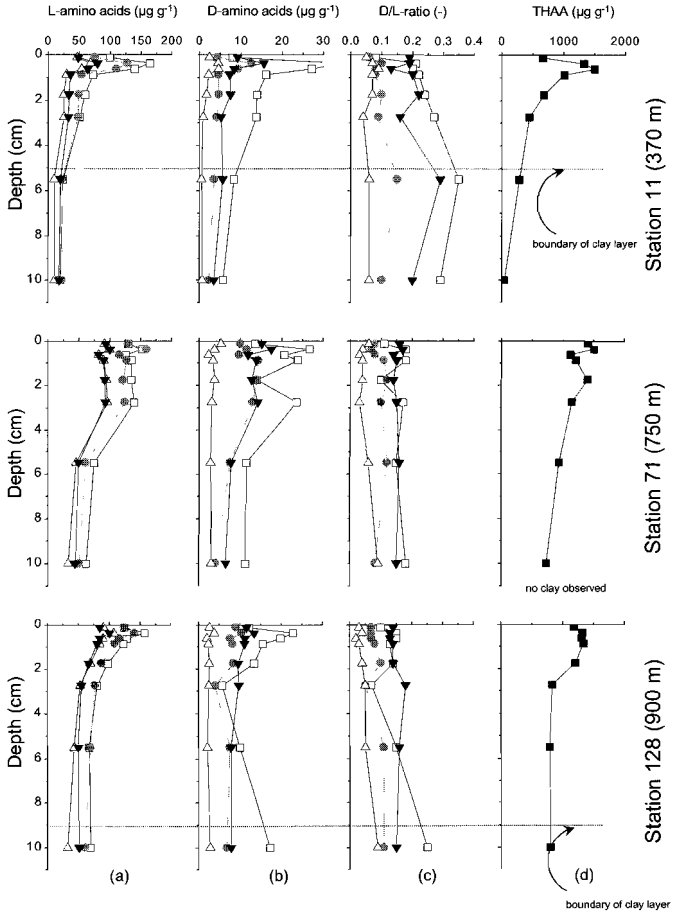

Figure 5.6 Concentration of L-amino acids (a), D-amino acids (b), D/L-amino acid ratios (c), and THAA concentrations (d) in the upper 10 cm of sediments at stations 11 (370 m), 71 (750 m), and 128 (900 m) across the FSC slope. DL-aspartic acid, glutamic acid, serine and alanine are shown by, respectively, □, ●, △, ▼.

Discussion

Distribution of D-amino acids across the slope

Earlier data on TOC, TN and THAA in suspended matter, sediment trap samples and surface sediments from the PROCS 99-1 cruise indicated a mid-slope accumulation of young, amino acid-rich organic matter (Grutters et al., 2001c). From a simple end-member

model they concluded that organic matter (OM) settling directly from the euphotic layer contributed little (~20%) to OM in near-bottom sediment traps. Furthermore, it was suggested that the mid-slope amino acid accumulation was caused by down-slope transport of OM in near-bottom suspended matter layers. During events of increased bottom currents coarse, OM-poor particles were resuspended from the sediment surface.

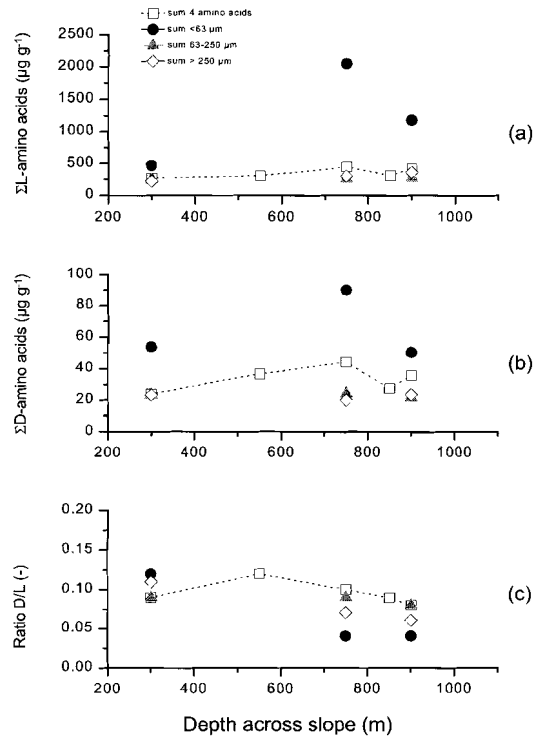

Figure 5.7 Concentration of the sum of L-amino acids (a) and D-amino acids (b) and the ratio of D/L-amino acids (c) in the sediment surface (0-2.5 mm) at stations 11 (370 m), 61 (550 m), 71 (750 m), 68 (850 m) and 128 (900 m) across the slope of the FSC. The concentrations are given for the bulk sediment (□), the <63 µm fraction (●), the 63-250 µm fraction (▲) and the >250 µm fraction (◇).

By using a similar end-member model we will evaluate whether the distribution of D-amino acids is correlated to that of THAA across the FSC slope. The end-member model is based on suspended matter samples from the upper water column (station 61; 250 m water depth), near-bottom water column (station 61; 10 mab), sediment trap samples (C6) and size fractions of the sediment top layer (station 71). First, it was calculated whether the concentration of D-amino acids in near-bottom suspended matter samples (~10 mab) could be mimicked by mixtures of two end-members: (1) suspended matter from the upper water column and (2) fine particles (<63 µm) from the surface sediment. Second, it was calculated whether mixtures of these end-members could describe the concentration of D-amino acids in the sediment trap samples from "low flux" periods (i.e. low resuspension). The average end-member mixture that best described both the suspended matter in the near-bottom layers and the "low flux" trap samples will be called 'fluff', *sensu* Bonnin et al.

(2001) (see Fig. 5.8). The next step was to estimate whether the composition of the "high flux" trap samples (i.e. increased resuspension) could be explained by mixtures of four end-members: (1) fluff, and the (2) <63 µm fraction, (3) 63-250 µm fraction and (4) the >250 µm fraction from the surface sediment. In first instance, the fluff was further diluted with additional fine particles. When the model calculated an addition of the coarser fractions, all fractions were added according to their relative abundance in the sediment surface, i.e. the sediment proper was resuspended in addition to the fluff. In the model the difference between the calculated concentrations of DL-amino acids from the end-member mixtures and the measured values in the samples were minimized by using the Excel solver routine (least-squares).

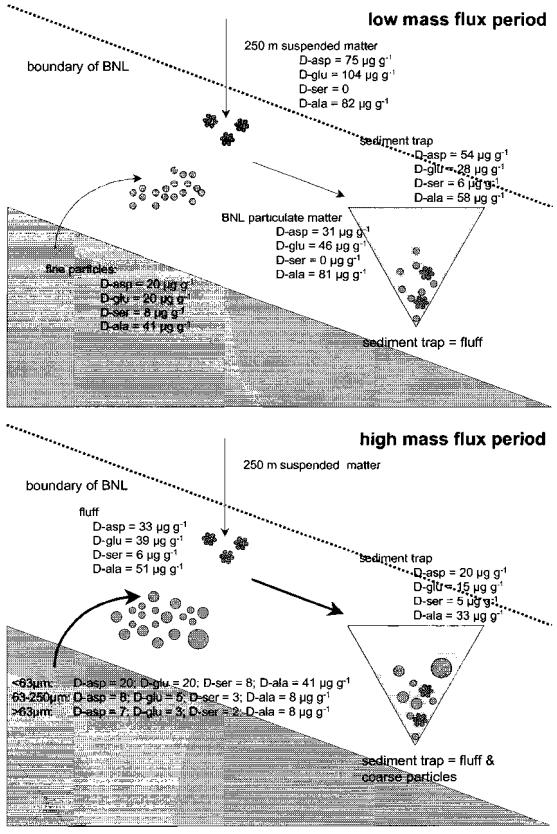

Figure 5.8 An end-member model based on D-amino acid distributions was used to estimate the composition of enantiomers in sediment trap samples and near-bottom suspended matter as a mixture of suspended matter from the upper water column (250 m depth) and fine particles from the sediment surface. This mixture is called fluff *sensu* Bonnin et al. (2001). During "low flux" periods the fluff at 750 m depth was estimated to consist of 77% of fine particles, resuspended from the seabed, and 23% of suspended matter (250 m). D-amino acids in sediment traps during "high flux" periods (i.e. increased resuspension) was modeled as a mixture of 32% fluff and 68% of sediment particles (23% of additional <63 µm, 41% of 63-250 µm and 4% >250 µm).

A mixture of 77% D-amino acids from the finest (<63 µm) fraction of the sediment top layer and 23% D-amino acids from the upper water column explained both the composition

of D-amino acids in near-bottom suspended matter and in the sediment trap (Fig. 5.8). The "high flux" sediment trap material was estimated to contain 32% fluff and 68% sediment particles (23% <63μm, 41% 63-250 μm and 4% >250 μm) due to increased resuspension (Fig. 5.8). The results demonstrate that D-amino acids in aggregates settling from the upper water column are not the major source of D-amino acids in near-bottom layers, and that most come from fine particles resuspended from the sediment surface. The fine particles appear to be present in near-bottom layers during periods of both low and high resuspension. The lower concentration of D-amino acids during high resuspension periods can be explained by addition of coarser sediment particles. These conclusions are strongly supported by the observation that D-ser occured in the sediments but was not present in suspended matter (Fig. 5.2). The presence of D-ser in the sediment traps must, therefore, be explained by resuspension from the sediment surface (Fig. 5.6). These results are in good agreement with those of a similar end-member model based on THAA, TN and amino acid mole-percentages (Grutters et al., 2001c) and an end-member model based on total mass flux and TOC (Bonnin et al., 2001). Hence, we conclude that the erosion-deposition mechanism that is responsible for the distribution of THAA across the slope is responsible also for the distribution of D-amino acids.

Contribution of D-amino acids in the sediments by racemization

With increasing age, a-biotic racemization of L- into D-amino acids will increasingly contribute to the concentration of D-amino acids. The racemization rate is different for each individual amino acid, and depends on pH and temperature. Racemic mixtures, with equal amounts of L-and D-amino acids, will be reached on time scales of 10^5-10^6 years. The age of OM in aggregates in the water column is on the order of days to weeks, considering the sinking rates of 100-200 m d^{-1} (Honjo et al., 1982a; Fowler and Knauer, 1986). Therefore, OM in aggregates is too young for any contribution of D-amino acids by racemization. The age of the OM in suspended matter during down slope transport in bottom nepheloid layers is probably on the order of months to years (e.g. Epping et al., 2001), which is still too young for any contribution by racemization. However, with sedimentation rates of ~1 cm ky^{-1} the age of the OM in the sediments may become sufficient for a significant contribution of D-amino acids by racemization.

We will evaluate the contribution of racemization to the D-amino acids in the sediment at station 71 (750 m). The transition from the clay layer to overlying coarser sediments, that was interpreted as the transition from glacial to interglacial conditions (Stoker et al., 1991), was not observed in the upper 10 cm. This implies that the age is 10,000 years at most. The contribution of D-amino acids by racemization was estimated by application of the 'sedimentation-sediment mixing-degradation-racemization' model that was described in detail by Grutters et al. (2001b). We used similar racemization rates as calculated in their study (2001b). The sedimentation rate ω was estimated as 0.001 cm y^{-1}. The sediment mixing coefficient D_b of 0.06 cm^2 y^{-1} was obtained from fitting ^{210}Pb profiles. The amino acid degradation rate k (y^{-1}) was estimated as 0.5 y^{-1}.

According to our model, the contribution of D-amino acids by racemization explained on average 8% of D-aspartic acid, 8% of D-glutamic acid, <1% of D-serine and 4% of D-alanine in the upper 10 cm of station 71 (750 m) (Fig. 5.9). We assumed equal degradation rates for both L- and D-amino acids, which may be incorrect since D-amino acids are degraded less efficiently than L-amino acids (Hopkins and Ferguson, 1994;

O'Dowd and Hopkins, 1998). However, when the degradation rates were lowered to 0.05 y^{-1} the contribution by racemization increased only a few percent (Fig. 5.9). The racemization rates that were used in the model were derived from experiments with free amino acids. Bada and Schroeder (1975) suggested that in aqueous solutions racemization rates of peptide-bound amino acids are 2-3 times higher than rates of free amino acids. However, increasing the racemization rates with a factor 3 enlarged the average contribution of racemization only to 12% for D-aspartic acid, with a maximum of 37% at 10 cm sediment depth. For D-glutamic acid, D-serine and D-alanine there was little effect on the estimated contribution of racemization. We conclude that there is a minor contribution to the total amount of D-amino acids by racemization and, hence, that there must be a considerable additional source of D-amino acids in the sediments to explain our measured concentrations.

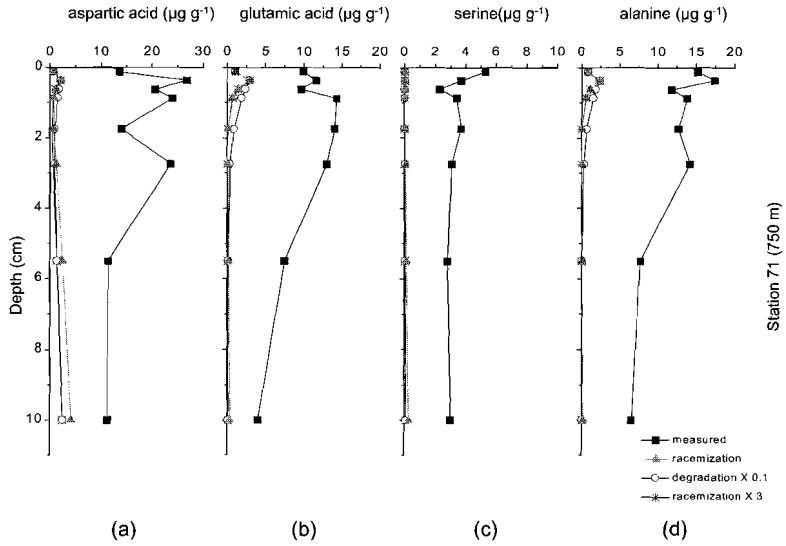

Figure 5.9 Results from the diagenetic 'sedimentation-sediment mixing-racemization-degradation' model for aspartic acid (a), glutamic acid (b), serine (c) and alanine (d) in the upper 10 cm of sediments at station 71. The results clearly indicate that the contribution of D-amino acids by racemization (∗) to the measured concentration of D-amino acids in the sediments (■) was negligible. Lower degradation rates (○) or increased racemization rates (▲) did not influence the modeling results.

Amino acid enantiomers versus organic matter ageing.

During incubation experiments with dissolved organic matter (DOM) degradation, Jørgensen et al. (1999) and Amon et al. (2001) observed that the D/L-ratios of amino acids increased from low values in fresh DOM to higher values in more diagenetically altered DOM. They proposed that the relative enrichment of D-amino acids during DOM degradation was accompanied by the production of relatively refractory bacterial cell walls, and that L-amino acids, mainly present in labile proteins, are rapidly degraded. We will further evaluate the character of the DL-amino acids in the FSC by plotting D/L-ratios against mole-percentages of non-protein amino acids, which indicate microbial degradation

(Whelan, 1977; Lee and Cronin, 1982). D/L-ratios as well as mole-% of non-protein amino acids are low in suspended matter samples, indicating the freshness of OM in the water column (Fig. 5.10). The D/L-ratio in the suspended matter was similar to that in the sediment traps. However, the mole-% of the non-protein amino acids was higher in the traps. Apparently, the amino acids in the sediment traps were partly degraded but still have low D/L-ratios. The D/L-ratios as well as mole-% of non-protein amino acids in the <63 µm fraction of station 71 (750 m) and 128 (900 m) are similar to those in the traps, confirming that this finest fraction contributed significantly to total mass fluxes in the traps. The relatively low D/L-ratios and mole-% of non-protein amino acids in the finest fractions at station 71 and 128, relative to the values in the bulk sediment at these depths illustrates the freshness of OM in this fraction. The increasing D/L-ratios and mole-% of non-protein amino acids from the sediment top layer to the deeper sediments suggests the increasing OM ageing and the concomitant enrichment of bacterial cell walls with depth in the sediments.

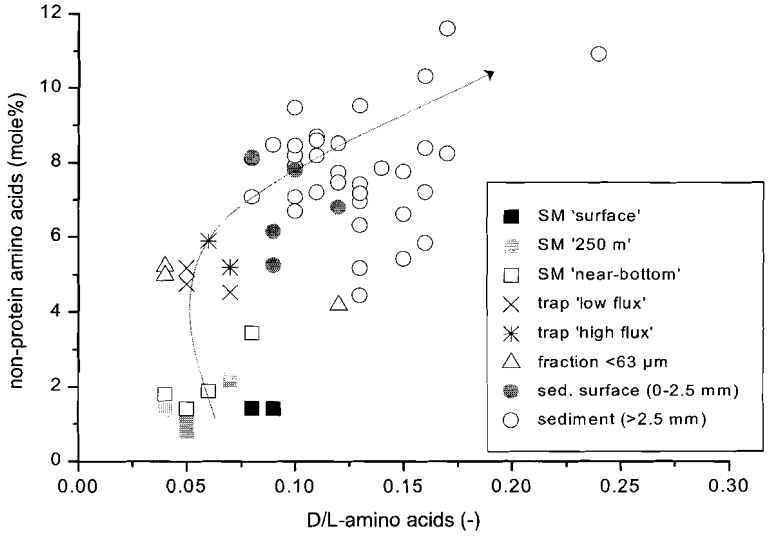

Figure 5.10 The relation between the D/L-amino acid ratio and mole-percentages of non-protein amino acids in samples from suspended matter in the water column, sediment traps, sediments, and in the <63 µm fraction of the sediment top layer (0-2.5 mm) across the slope of the FSC. The D/L-ratios and contributions of non-protein amino acid clearly increase from the suspended matter samples to the trap samples and then to the sediments (as indicated by the arrow). This illustrates that with increasing age of the organic matter, and hence with increasing degradation, there is an accumulation of D-amino acids relative to L-amino acids, which is likely to be associated with the accumulation of relatively refractory bacterial cell walls.

The contribution of bacterial cell walls to THAA in the sediments across the FSC

Considering the almost negligible contribution by racemization in our samples, the D-amino acids may be derived from bacteria, either from whole cells or from cell wall remnants. D-amino acids are present in peptidoglycans, the major constituent of bacterial cell walls. Four or five different types of peptidoglycans are known, differing in their types of cross-linking (Rogers, 1983). D-alanine is always present, and D-glutamic acid, D-

aspartic acid and D-serine are present depending on the type of peptidoglycan (Rogers, 1983; Meister, 1965; Pollock and Kvenvolden, 1978). In order to estimate the contribution of amino acids associated with peptidoglycans in bacterial cell walls (AA_{PG}) we used D/L ratios from pure peptidoglycan, as reported by Pedersen et al. (1999) and by Amon et al. (2001) (Table 5.1). Since Amon et al. (2001) did not report D/L-serine, we used the ratios from Pedersen et al. (1999). In addition, we derived D/L-ratios from Schleifer and Kandler (1972) for the types of peptidoglycan that contained all four D-amino acids (Table 5.1). The potential contribution of amino acids from whole cells (AA_{WC}) to THAA was estimated by using D/L-ratios reported for *S. Bacillaris* (McCarthy et al., 1998), for living cultures of bacterial strains *At, B* and *BJ* (Pedersen et al., 1999), and for arctic bacteria (Amon et al., 2001). Pedersen et al. (1999) measured ratios in two Gram-positive and a Gram-negative species. Here, an average D/L-ratio for the three species was calculated by taking into account that 70-90% of the bacteria is Gram-negative in marine sediments (Moriarty and Hayward, 1982). McCarthy et al. (1998) and Amon et al. (2001) do not give ratios for serine, which is therefore taken from Pedersen et al. (1999). The D/L-ratios and their references are given in Table 5.1. The possible contribution of AA_{PG} and AA_{WC} to THAA was calculated according $\Sigma_i\ [D\text{-}AA_i+(D\text{-}AA_i*L/D_i)]*1.2\ /\ THAA*100$. The factor 1.2 accounts for amino acids, other than the four D-amino acids, that are present in peptidoglycan (Schleifer and Kandler, 1972). The concentration of D-amino acids in the sediments was lowered for the contribution by racemization; for station 11 and 128 where no racemization was calculated the concentrations of the D-amino acids were lowered with the calculated contribution for station 71 (D-aspartic acid 8%, D-glutamic acid 8%, D-serine 1%, D-alanine 4%). We focus on the upper 5 cm to avoid discontinuities in age between the deeper sediment originating from glacial deposits and the upper sediment originating from the early Holocene (Stoker et al., 1991).

Table 5.1 Ratios of D/L-amino acids as measured in cultures of living bacteria and in (commercially available) peptidoglycan.

Sample	D/L-asp	D/L-glu	D/L-ser	D/L-ala	Reference
S. Bacillaris	0.14	0.09		0.38	McCarthy et al. (1998)
At. / B / BJ.	0.06	0.10	0.01	0.06	Pedersen et al. (1999)
Arctic bacteria	0.12	0.09		0.13	Amon et al. (2001)
Peptidoglycan	3.33	0.14	0.25	0.63	Schleifer (1972)
Peptidoglycan	0.29	0.47	0.03	0.33	Pedersen et al. (1999)
Peptidoglycan	0.30	0.49		0.44	Amon et al. (2001)

In the suspended matter samples the contribution of bacterially derived amino acids to THAA decreased from the ocean surface towards the seabed, except at station 128 (900 m) where the contribution was more constant with water depth. On average, 9 to 25% of the THAA in the suspended matter could be accounted for by amino acids from, respectively, cell walls and whole cells (Fig. 5.11a). In the "low flux" trap samples the contribution was increased to 10 (AA_{PG})-33% (AA_{WC}), and in the "high flux" samples even to 14-52%. In the sediments the contribution varied on average between 23-96%. The calculated AA_{WC}

exceeded the measured THAA at station 11 and 71 (Fig. 5.11b,c), indicating that at least part of the D-amino acids are associated with cell wall remnants and not with whole cells.

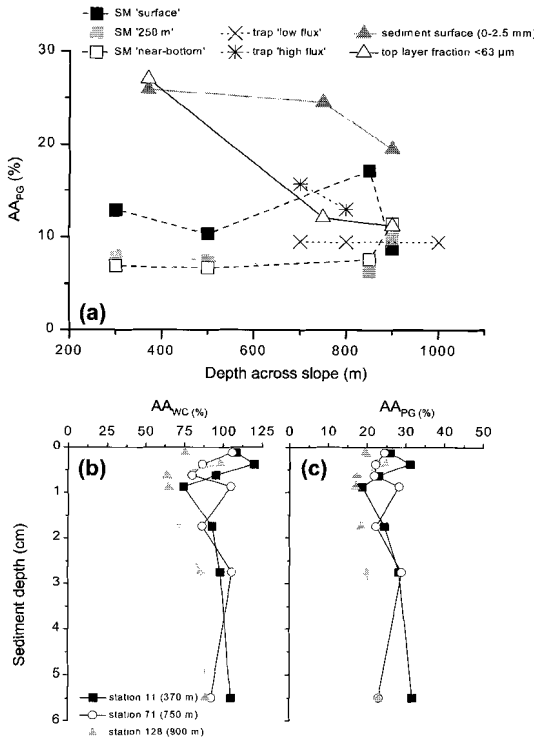

Figure 5.11 The contribution of amino acids from bacterial cell wall peptidoglycans (AA_{PG}) to THAA for suspended matter samples from station 78 (300 m), 61 (550 m), 71 (750 m) and 128 (900 m) (a). Suspended matter samples were collected from the surface (■), 250 m water depth (▩) and from near-bottom layers (10 mab) (□). Also shown are samples from "high" (✳) and "low flux" (×) sediment traps and bulk (▲) and size-fractionated (Δ) sediment samples (0-2.5 mm). Panels (b) and (c) show respectively the contribution of amino acids from whole cells (AA_{WC}) and AA_{PG} to THAA in the upper ~5 cm of the sediments across the FSC.

The contribution of amino acids from whole cells and/or cell wall remnants to THAA was further examined by inventories of L-and D-amino acids in the sediments across the FSC. The inventories, shown as the sum of the four individual amino acids, were calculated with sediment porosities that ranged between 48% (370 m) and 58% (850 m), and a mineral particle density of 2.65 g cm^{-3}. The inventory of L- and D-amino acids was similar across the slope (Fig. 5.12) when the upper 2 cm of the sediments were considered, but the inventory at station 71 (750 m) was much larger than that at the other stations when the upper 5 cm of the sediments were considered. This indicates that the enantiomers are mostly present in upper sediment layers, except at station 71 where the enantiomers accumulated to the deeper sediment. The D/L-amino acid ratio, derived from the inventories, decreased with depth across the slope. This suggests that the contribution of cell wall remnants, rather than whole cells, are relatively important at the upper slope and that deeper down-slope there is a larger contribution of whole cells. Therefore, during

down-slope transport of fine, labile organic matter-rich particles new cells, with relatively low D/L-amino acid ratios, may be generated that accumulate at 750 m depth. The plot showing the relation between D/L-amino acid ratios and mole-% of non-protein amino acids illustrated that with increasing depth in the sediments these amino acids are preserved as cell wall remnants, rather than whole cells.

Although the contribution of cell wall (remnant) amino acids in the sediments was rather constant between the stations across the slope (Fig. 5.11c), the mid-slope accumulation of D-amino acids resulted in an elevated concentration of bacterial cell walls at this depth (Fig. 5.13). Rogers (1983) reported that amino acids in bacterial cell walls are resistant to many common hydrolytic enzymes and that their contribution to THAA will increase with increasing OM age. Knicker et al. (2001) proposed that remains of peptidoglycans were incorporated in insoluble geopolymers. Therefore, the transformation of degradable amino acids into refractory bacterial cell walls could be the first step in the production of hydrophobic geopolymers and, hence, in the long-term burial of amino acids in marine sediments.

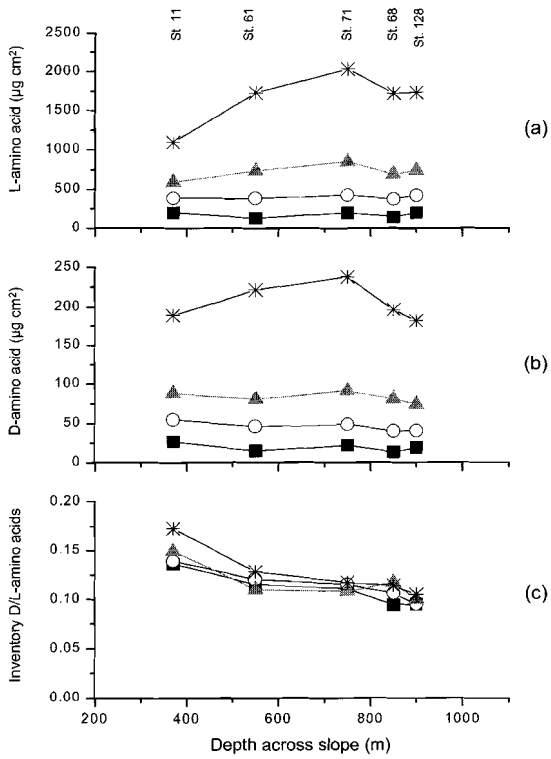

Figure 5.12 Inventories (µg cm^{-2}) of L-amino acids (a), and D-amino acids (b) in the sediments at station 11 (300 m), 61 (550 m), 71 (750 m), 68 (850 m), and 128 (900 m) across the FSC slope. The inventories of L- and D-amino acids integrated over the upper 0.5 cm of the sediment (■), upper 1 cm (○) or upper 2 cm (▲) were similar across the slope, and indicated that in the shallow sediment the accumulation of L- and D-amino acids is constant across the slope. Considering the upper 5 cm (✳), however, there was a much stronger accumulation at station 71.

The ratio of D/L-amino acids in the upper 5 cm of the sediment, obtained from the inventories (c), decreased with depth across the slope.

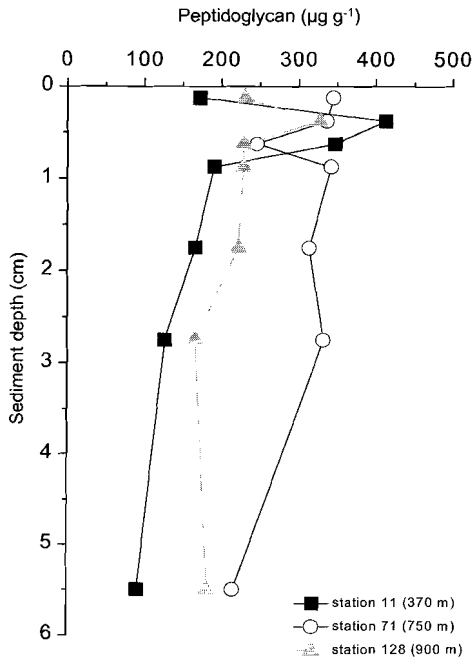

Figure 5.13 The concentration of amino acids derived from bacterial cell wall peptidoglycans in the upper ~5 cm of the sediments across the FSC slope.

Conclusions

1 From our end-member model, based on D-amino acids in suspended matter, sediment traps and surface sediments, it was concluded that the distribution of amino acid enantiomers across the FSC is correlated to the distribution of THAA. D-amino acids in settling aggregates from the upper water column were not the major source of D-amino acids in near-bottom layers and in the sediments.

2. On average, approximately 5% of the measured concentration of D-amino acids was accounted for by racemization.

3. During down-slope transport of fine, labile organic matter-rich particles an increasing proportion of amino acids from THAA is associated with, newly generated, whole bacterial cells that accumulate at 750 m depth. With increasing depth in the sediments these amino acids are preserved as cell wall remnants and contribute at least 24% to THAA in the deeper sediments.

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