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Structural variability of the neutral carbohydrate moiety of cow colostrum κ-casein as a function of time after parturition

Identification of a tetrasaccharide with blood group I specificity

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New neutral oligosaccharides from cow colostrum κ-casein were identified and characterized by 500-MHz ^{1}H-NMR spectroscopy. Their structures are Galβ(1→3)GalNAc-ol, Galβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol, Galβ(1→3)[Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol, Galβ(1→3){Fucα(1→3)[Galβ(1→4)]GlcNAcβ(1→6)}GalNAc-ol. The tetrasaccharide and the cow colostrum κ-caseinoglycopeptide which contains this oligosaccharide inhibit the hemagglutination of blood group I human erythrocytes. In cow mature milk only the disaccharide is characterized. The variability of these neutral oligosaccharides in cow κ-casein as a function of time after calving is studied.

κ-Casein is the unique sugar-containing casein fraction. The carbohydrate chains of mature cow milk κ-casein are constituted of three different oligosaccharides: NeuAcα-(2→3)Galβ(1→3)GalNAc-ol, Galβ(1→3)[NeuAcα(2→6)]GalNAc-ol and NeuAcα(2→3)Galβ(1→3)[NeuAcα(2→6)]GalNAc-ol [1, 2]. Cow colostrum and cow mature κ-caseins have the same peptide part; however the colostrum protein has a higher oligosaccharide content [3] with two additional sugars, i.e. *N*-acetylglucosamine and fucose, and its sugar moieties are much more complex [4, 5]. Six different oligosaccharides [6–9] have already been identified. With the exception of the tetrasaccharide Galβ(1→3)[Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol, they contained NeuAc [6–9].

New neutral oligosaccharides from cow colostrum κ-caseinoglycopeptide samples (C-terminal part of cow κ-casein, residues 106–169, containing all the sugars) obtained 15 min, 18 h and 42 h after calving are described. While only one neutral disaccharide Galβ(1→3)GalNAc-ol was identified in mature milk κ-caseinoglycopeptide which had no blood group I specificity, four neutral oligosaccharides (from di- to pentasaccharide) were characterized by 500-MHz ^{1}H-NMR spectroscopy in cow colostrum κ-caseinoglycopeptide. The latter and the tetrasaccharide which it contains inhibited the hemagglutination of I-positive human erythrocytes. Immunological experiments seem thus to rule out the possibility that the short sugar groups observed just after calving are split products arising from longer prosthetic oligosaccharides.

MATERIALS AND METHODS

κ-Caseinoglycopeptide preparation

Cow colostrum κ-caseinoglycopeptide was prepared according to [3] from the colostrum of a unique cow obtained 15 min, 6 h, 18 h, 30 h, 42 h and 60 h after parturition. Mature cow milk κ-caseinoglycopeptide was prepared according to [10].

β-Elimination

The κ-caseinoglycopeptide (75 mg) was treated with alkaline borohydride (0.05 M NaOH and 1.0 M $NaBH_4$) for 18 h at 45 °C under nitrogen in the dark [11]. After desalting on Dowex 50 W-X_2 (H^+) with 2 mM formic acid as eluent and washings with methanol, the oligosaccharide-alditols were isolated by filtration on Bio-Gel P_4 (250 × 0.9 cm) (Bio-Rad) with water as eluent. Sugar-positive fractions stained with the orcinol/sulfuric acid reagent were further purified by HPLC.

HPLC of oligosaccharide-alditols

HPLC of oligosaccharide-alditols was conducted on a Gilson chromatograph equipped with a Micro Pak AX-5 column (30 × 0.4 cm) (Varian). Elution was performed with a linear gradient of acetonitrile/water (85:15–30:70, v/v) for 90 min at room temperature and a flow rate of 1 ml/min. All solvents were degassed by sonication, before application. Sugars were detected at 200 nm.

Analytical methods

Galactose was determined following Schultze et al. [12] after acid hydrolysis (1 M HCl, 3 h, 110 °C), sialic acid by Warren's method [13] after acid hydrolysis (0.1 M HCl, 1 h, 80 °C). GalNAc and GlcNAc were characterized using a Biotronik amino acid autoanalyzer, model LC 6000.

The carbohydrate composition was established by gas-liquid chromatography after methanolysis in methanol/0.5 M HCl at 80 °C for 24 h and re-*N*-acetylation with acetic anhydride during 16 h at 20 °C, washings with hexane and trimethylsilylation with 20 µl *N,O*-bis(trimethylsilyl) trifluoro-

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acetamide (Pierce) and 10 µl pyridine (Merck) (1 h at 20°C). Gas chromatography was performed with a Hewlett-Packard 5710 A instrument modified with a 18740 B capillary column control equipped with a capillary column (2600 × 0.023 cm) containing CP Sil 5 CB on WCOT Fused Silica (Chrompack). Mannitol was used as an internal reference and a mixture of free sugars as standards. Oven temperature was increased linearly from 120°C to 250°C at a rate of 2°C per min.

500-MHz ^{1}NMR spectroscopy

Oligosaccharide-alditols were repeatedly exchanged in ^2H_2O (99.96% ^{2}H, Aldrich) with intermediate lyophilization. ^{1}H-NMR spectra were recorded on a Bruker AM-500 spectrometer (SON hf-NMR facility, Department of Biophysical Chemistry, University of Nijmegen, The Netherlands) operating at 500 MHz in the Fourier transform mode at a probe temperature of 27°C. Resolution enhancement of the spectra was achieved by Lorentzian-to-Gaussian transformation [14]. Chemical shifts are given relative to sodium 4,4-dimethyl-4-silapentane-1-sulfonate but were actually measured indirectly to acetone in ^2H_2O (δ = 2.225 ppm) [15].

Blood group antibodies and human erythrocytes

The murine monoclonal anti-I (M 18.3) was a generous gift from Dr P. A. W. Edwards from the Ludwig Institute for Cancer Research (London Branch, UK) and the human anti-I (Ma) and anti-i (Den) sera were donated by Dr M. C. Crookston from the Toronto General Hospital (Toronto, Ontario). The specificity of these antibodies has been described elsewhere [16, 17].

Red cells from adult group OI$^+$ donors were from the Centre National de Transfusion Sanguine, Paris. For use in agglutination or binding assays the cells (1 vol. of a 6% suspension) were fixed for 30 min at 4°C with 0.6% (v/v) glutaraldehyde in NaCl 150 mM (2 vol.) and washed six times or were papain-treated as described before [18].

Inhibition of agglutination and radiobinding assays

Inhibition agglutination assays using the anti-I and anti-i antibodies are described in the legend to Table 5 using papain-treated erythrocytes.

For the radiobinding assay, the human anti-I (Ma) antibody was first affinity purified by a fixation-elution technique with glutaraldehyde-fixed adult OI$^+$ red cells. The antibody recovered in the heat eluate was ^{125}I-labelled by the iodogen protocole [19] to a specific activity of 2×10^6 cpm/µg protein. More than 95% of the radiolabelled material was precipitable by trichloroacetic acid. Detailed protocol for the radiobinding assay using glutaraldehyde-fixed papain-treated erythrocytes is described in the legend to Fig. 3.

RESULTS

Sugar analysis of the κ-caseinoglycopeptides

The detailed quantitative sugar analyzes of cow colostrum κ-caseinoglycopeptides obtained 15 min, 6 h, 18 h, 30 h, 42 h and 60 h after parturition, and of mature cow milk κ-caseinoglycopeptide are indicated in Table 1. The sugar content of the κ-caseinoglycopeptide obtained just after parturition was high when compared to the normal mature peptide and decreased to a normal level 60 h after parturition; GlcNAc disappeared after 66 h.

Table 1. *Composition of the sugar moiety of cow colostrum κ-caseinoglycopeptides obtained after different time intervals after parturition and of mature cow milk κ-caseinoglycopeptide*

κ-Caseino-glycopeptide	Time after parturition	Gal	GlcNAc	GalNAc	NeuAc
	h	%			
Colostrum	0.25	18.4	1.6	5.3	9.9
Colostrum	6	16.2	1.4	4.7	10.5
Colostrum	18	13.4	0.9	3.2	4
Colostrum	30	16.3	1.2	4.2	4
Colostrum	42	14.1	0.9	3.6	4.3
Colostrum	60	13.5	0.3	1.4	1.4
Mature milk	>66	4	0	5.5	6.5

Separation of oligosaccharide-alditols of cow colostrum and mature milk κ-caseinoglycopeptides by HPLC

Cow colostrum and mature cow milk κ-caseinoglycopeptides (75 mg) were submitted to alkaline borohydride treatment and subsequently filtered on Bio-Gel P_4. The first fractions contained peptides, the middle fractions carbohydrate-rich material and the last fractions salts. The fractions with low-molecular-mass carbohydrate material were further fractionated by HPLC. The elution profiles are shown in Fig. 1. Four major oligosaccharide-alditols (A_1 to A_4) were studied from cow colostrum κ-caseinoglycopeptide A obtained 15 min after parturition whereas three (B_1 to B_3 and C_1 to C_3) were analyzed when the κ-caseinoglycopeptides obtained 18 h (B) and 42 h (C) after parturition were studied. Only one oligosaccharide alditol (D_1) was obtained from mature cow milk κ-caseinoglycopeptide D. The carbohydrate content of each oligosaccharide-alditol and the molar carbohydrate composition of the various HPLC-separated oligosaccharide fractions are reported in Table 2.

The oligosaccharide-alditol fractions obtained after HPLC separation were subjected to 500-MHz ^{1}H-NMR spectroscopy. Four different neutral compounds and one acidic compound (see Scheme 1) could be identified by comparison of the ^{1}H-NMR data with those of a set of reference compounds [9, 20]. The chemical shifts of the structural reporter groups of the various compounds as occurring in the fractions $C_1$and A_4 together with those of the appropriate reference compounds are presented in Table 3. As is evident from Table 4 most of the fractions contain more than one oligosaccharide-alditol. The ratios of the constituents in the mixtures were derived from the intensities of the signals in the ^{1}H-NMR spectra.

Fig. 2 shows the oligosaccharide content of cow colostrum κ-casein characterized 15 min, 18 h and 42 h after calving.

Blood group activity of κ-caseinoglycopeptides and oligosaccharide-alditol fractions

The Ii blood group activities of κ-caseinoglycopeptides fractions A, B, C and D obtained at various intervals of time after parturition using an hemagglutination inhibition technique are shown in Table 5. We found that fractions B and C were good inhibitors of the murine monoclonal anti-I

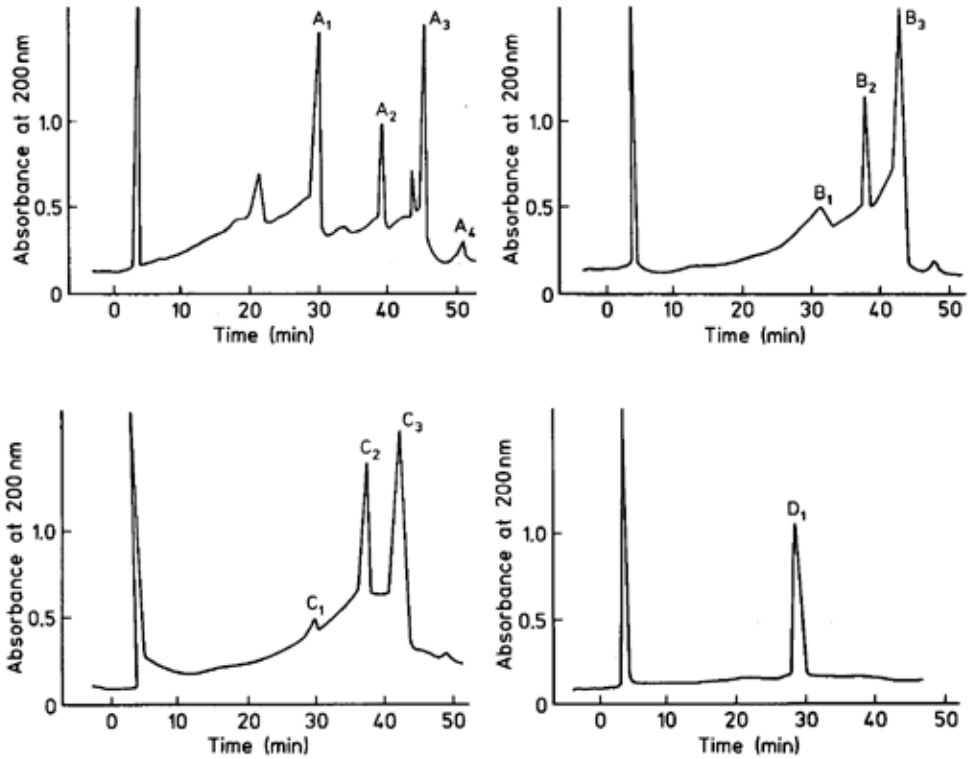


Fig. 1. *HPLC of oligosaccharide-alditols obtained from cow colostrum and mature cow milk κ-caseinoglycopeptides on a MicroPak AX-5 column (30 × 0.4 cm) (Varian).* Elution conditions: a 90-min linear gradient of 85:15 – 30:70 (v/v) acetonitrile/water. Absorption was determined at 200 nm. (A) Oligosaccharide-alditols from cow colostrum κ-caseinoglycopeptide obtained 15 min after parturition; (B) oligosaccharide-alditols from cow colostrum κ-caseinoglycopeptide obtained 18 h after parturition; (C) oligosaccharide-alditols from cow colostrum κ-caseinoglycopeptide obtined 42 h after parturition; (D) oligosaccharide-alditols from mature cow milk caseinoglycopeptide

Table 2. *Carbohydrate composition of oligosaccharide-alditols derived from cow colostrum and mature cow milk κ-caseinoglycopeptides*
Molar ratios of monosaccharides were calculated on the basis of one residue of GalNAc-ol

HPLC fraction	Time after parturition	Fuc	Gal	GlcNAc	GalNAc-ol
	h	mol/mol			
Colostrum					
A1	0.25	0	1.0	traces	1.0
A2	0.25	0	1.1	0.4	1.0
A3	0.25	0	2.1	0.6	1.0
A4	0.25	traces	1.4	0	1.0
B1	18	0	0.9	0.5	1.0
B2	18	0	1.0	0.5	1.0
B3	18	0	1.4	0.7	1.0
C1	42	0	1.5	0.8	1.0
C2	42	0	2.0	1.0	1.0
C3	42	0	2.0	1.0	1.0
Mature milk					
D1	>66	0	1.0	0	1.0

antibody (M 18.3) whereas fractions A and D were poorly active. None of the fractions inhibited the human polyclonal anti-i (Den) nor human blood group anti-A and anti-B sera (not shown). Prior desialylation of glycopeptides by treatment with neuraminidase from *Vibrio cholerae* (12.5 mU for 18 h at 37 °C) does not significantly modify these results.

The blood group I and i activity of some oligosaccharide-alditols prepared from the κ-caseinoglycopeptides has been investigated by a radiobinding assay using the affinity purified ^{125}I-labelled anti-I(Ma) antibody. Preliminary assays indicated that among the κ-caseinoglycopeptides only glycopeptides B and C were inhibitors in this system (0.2 – 0.4 mg/ml, data not shown), thus confirming the blood group I activity of these fractions demonstrated with the murine monoclonal antibody (Table 5). However the glycopeptides inhibited the murine monoclonal antibody better than the human polyclonal antibody, presumably since the former might recognize a smaller epitope.

As shown from Fig. 3, the oligosaccharide-alditols B_3 and C_3 were equally good inhibitors of anti-I(Ma), whereas oligosaccharide A_1 was not: these results correlate well with those obtained above using the native κ-caseinoglycopeptides. The inhibitory concentration for 50% inhibition was almost identical for oligosaccharides B_3 and C_3 (1.3 and 1.8 mM, respectively) and is in the same range as observed with other oligosaccharides obtained either by chemical or enzymatic synthesis [18].

DISCUSSION

The carbohydrate moiety of κ-casein during the colostral period is characterized by the presence of *N*-acetylglucosamine which disappears 66 h after calving [3]. In the present study we investigated neutral oligosaccharide-alditols isolated from cow colostrum and mature milk κ-caseins and their

Table 3. *^1H chemical shifts of structural reporter-groups of constituent monosaccharides of the cow colostrum κ-caseino-glycopeptide neutral oligosaccharide-alditols*
The chemical shifts of fractions C1 and A4 are compared to literature values R1 [20] and R2 [9]

Residue	Reporter group	Chemical shift in									
		Galβ-(1→3)GalNAc-ol		Galβ(1→3)-[GlcNAcβ(1→6)]-GalNAc-ol		Galβ(1→3)-[Galβ(1→4)-GlcNAcβ(1→6)]-GalNAc-ol		NeuAcα(2→3)-Galβ(1→3)-GalNac-ol		Galβ(1→3)-{Fucα(1→3)-[Galβ(1→4)]-GlcNAcβ(1→6)}-GalNAc-ol	
		R1	C1	R1	C1	R1	C1	R2	A4	R1	A4
		ppm									
GalNAc-ol	H-2	4.395	4.391	4.395	4.391	4.394	4.391	4.390	4.390	4.393	4.390
	H-3	4.065	4.061	4.061	4.061	4.060	4.061	4.074	4.072	4.060	n.d.
	H-4	3.507	3.510	3.468	3.468	3.465	3.468	3.498	3.497	3.454	n.d.
	H-5	4.196	4.192	4.281	4.279	4.282	4.279	4.187	4.187	4.270	4.269
	NAc	2.050	2.050	2.066	2.066	2.067	2.066	2.046	2.046	2.067	2.067
Galβ(1→3)	H-1	4.478	4.478	4.468	4.465	4.465	4.465	4.547	4.546	4.463	4.464
	H-3	–	–	–	–	–	–	4.122	4.122	–	–
	H-4	3.901	3.902	3.901	3.902	3.900	3.902	3.931	3.930	3.899	n.d.
GlcNAcβ(1→6)	H-1	–	–	4.538	4.539	4.560	4.561	–	–	4.560	4.562
	H-6	–	–	3.932	3.93	3.998	3.998	–	–	4.010	4.010
	NAc	–	–	2.066	2.066	2.064	2.066	–	–	2.056	2.056
Galβ(1→4)	H-1	–	–	–	–	4.470	4.470	–	–	4.448	4.448
	H-4	–	–	–	–	3.925	3.927	–	–	3.927	n.d.
Fucα(1→3)	H-1	–	–	–	–	–	–	–	–	5.109	5.110
	CH3	–	–	–	–	–	–	–	–	1.174	1.174
NeuAcα(2→3)	H-3*ax*	–	–	–	–	–	–	1.800	1.800	–	–
	H-3*eq*	–	–	–	–	–	–	2.774	2.772	–	–
	NAc	–	–	–	–	–	–	2.034	0.034	–	–

structural variations during the colostral period. We established that the disaccharide Galβ(1→3)GalNAc-ol was present in cow colostrum κ-casein (42% of the total sugar amount) 15 min after parturition and then decreased very quickly and reappeared after 66 h as the unique neutral sugar. The tetrasaccharide Galβ(1→3)[Galβ(1→4)GlcNAcβ-(1→6)]GalNAc-ol was always present in cow colostrum κ-casein but its amount depends on the time after parturition: 15 min after parturition it represents 44.3% of the total sugar amount, after 18 h 89% and after 42 h 91.7%; it almost disappeared after 66 h. The trisaccharide Galβ(1→3)[GlcNAcβ-(1→6)]GalNAc-ol was always present in cow colostrum κ-casein but at a low ratio (around 10%). For the first time the possible presence of a pentasaccharide with fucose (Fig. 2) was identified in cow κ-casein.

κ-Casein has only *O*-glycosidic linkages but the number of the glycosylation sites varies. The potential number of carbohydrate binding sites lies between 0 and 5. GalNAc is situated on threonines 131, 133, 135 (or 136) and 142 and on serine 141 [4, 21–23] in mature and colostrum κ-caseins. According to Vreeman et al. [24], bovine κ-casein can be fractionated into ten components, each differing in NeuAc and/or phosphorus content(s). The carbohydrate-free fraction is about 34%, the sum of the fractions with one and two NeuAc groups (i.e. one GalNAc) 30%, the fractions with three or four NeuAc groups (i.e. two GalNAc) 18%, with five or six and more NeuAc groups (i.e. three or more GalNAc) 6%. These results suggest that glycosylation of cow κ-casein occurs by a random mechanism.

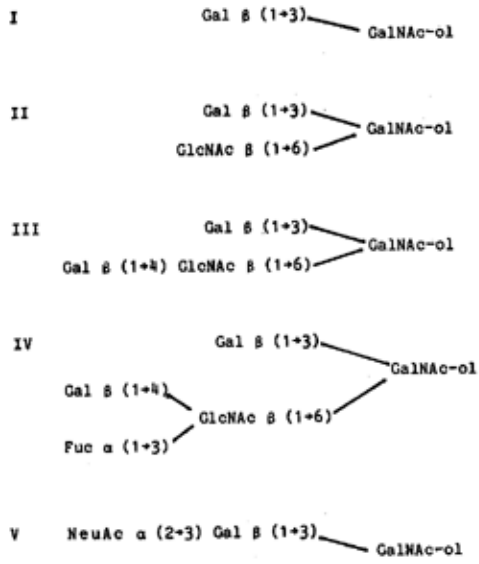
The oligosaccharide chains found in κ-casein are rather common constituents for mucin-type glycoproteins occurring in various organs and species [25]: it is remarkable that in general these carbohydrates exhibit such a heterogeneity. In view of the fact that many mucins are exposed to direct or indirect contacts with the environment, wherein the organism lives, it may be that the heterogeneity of the carbohydrates plays a role in furnishing protection against attacks from the outer world.

Only the κ-caseinoglycopeptides B and C possess blood group I (but not i) activity, suggesting the presence of branched chain oligosaccharides. The κ-caseinoglycopeptides A and D have no significant blood group I activity: this observation might be explained either by the absence or by the masking of I-reactive structures. Our structural studies with oligosaccharide-alditols isolated from κ-caseinoglycopeptides demonstrated that the first hypothesis is true. The immunological experiments performed with the oligosaccharide-alditols corroborate the results obtained with the κ-caseinoglycopeptides as the fractions B_3 and C_3 are the most active.

Sequence analysis shows that oligosaccharide-alditols B_3 and C_3 have identical structures and that they contain an *N*-acetyllactosamine unit (LacNAc) branched in β(1→6) on GalNAc of the disaccharide Galβ(1→3)GalNAc. This structure is different from those more commonly identified in which the LacNAc is branched in β(1→6) on residue Gal of a GlcNAcβ(1→3)Gal unit [26]. However it is well established that the sequence Galβ(1→4)GlcNAcβ(1→6) corresponds to

Table 4. *Sequence established by 500-MH_z ^{1}H-NMR of HPLC-separated neutral oligosaccharide-alditols derived from cow colostrum and mature cow milk κ-caseinoglycopeptides*

HPLC fraction	Relative mass of sequence					
	Galβ(1→3) GalNAc-ol	Galβ(1→3) GalNAc-ol GlcNAcβ(1→6)	Galβ(1→3) GalNAc-ol Galβ(1→4)GlcNAcβ(1→6)	Galβ(1→3) GalNac-ol Galβ(1→4) GlcNAcβ(1→6) Fucα(1→3)	NeuAcα(2→3)Galβ(1→3) GalNAc-ol	
	%					
Colostrum						
A1	85	5	10			
A2		45	55			
A3			100			
A4				20	80	
B1		20	80			
B2		40	60			
B3			100			
C1	33	22	45			
C2		30	70			
C3			100			
Mature milk						
D1	100					



Scheme 1. *Structure of the carbohydrate moieties obtained from cow colostrum κ-casein*

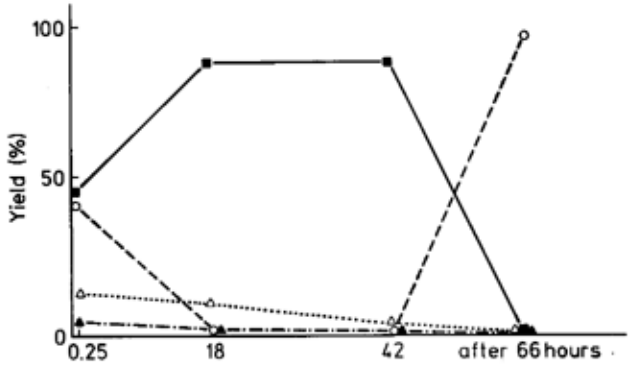


Fig. 2. *Variability of the amount of neutral oligosaccharides I–IV isolated from cow colostrum κ-casein (obtained 15 min, 18 h and 42 h after calving) and from mature cow milk κ-casein (obtained after 66 h after calving).* (○–––○) I; (△ · · · · △) II; (■——■) III; (▲–·–·–▲) IV. The structures of I–IV are shown in Scheme 1

Table 5. *Inhibition of hemagglutination by κ-caseinoglycopeptides obtained after different time intervals after calving*

Hemagglutination inhibition was assayed of a murine monoclonal anti-I antibody and of a human anti-i serum by the κ-caseinoglycopeptides. In the assay, 20 µl of the antibody at the appropriate dilution in 50 mM Tris/HCl, pH 8.0, 100 mM NaCl, 0.3% bovine serum albumin, containing 0.02% (w/v) sodium azide, was incubated with 20 µl of inhibitor diluted in the same buffer. After 1 h at 4°C, 20 µl suspension of 20% (v/v) papain-treated adult group OI+ erythrocytes was added and the mixture was further incubated for 1 h at 4°C. Agglutination was read under the microscope. The κ-caseinoglycopeptides are defined in the legend to Fig. 1

κ-Caseinoglycopeptide	Minimum concentration for 50% inhibition	
	anti-I (M18.3) (1:4000)	anti-i (Den) (1:3200)
	mg/ml	
A	3	>15
B	0.15	>15
C	0.075	>15
D	7.5	>15

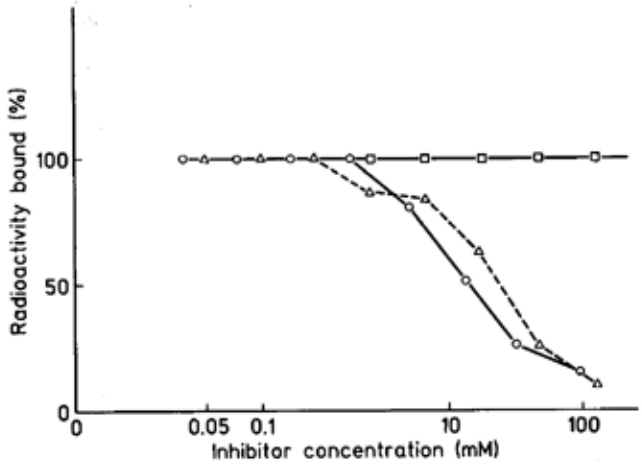


Fig. 3. *Inhibition of human anti-I (Ma) antibody by oligosaccharides from κ-caseinoglycopeptide.* The affinity purified ^{125}I-labelled anti-I(Ma) was adjusted to 250 µg/ml in 50 mM Tris/HCl, pH 8.0, 100 mM NaCl, 0.3% (w/v) bovine serum albumin containing 0.02% sodium azide; 10 µl of this solution was added to appropriate amounts of inhibitor pipetted into 0.4-ml Eppendorf tubes and lyophylized. After 1 h at 4°C, 10 µl of a suspension of glutaraldehyde-fixed, papain-treated adult OI+ erythrocytes were added (5×10^5 cells) and a further 1-h incubation at 4°C was carried out. After several washes in 150 mM NaCl, the amount of ^{125}I-labelled antibody bound to the red cells was determined by gamma counting. Oligosaccharide-alditols A_1 (□), B_3 (○) and C_3 (△)

the antigenic determinants recognized by the anti-[blood group I (Ma)] antibodies [27] and probably by the murine monoclonal anti-I antibody [17] although some differences might exist. These structures do not contain repetitive sequences [Galβ(1→4)GlcNAcβ(1→3)], explaining the absence of blood group i activity. These data are in agreement with previous studies indicating that anti-I and anti-i sera recognized oligosaccharide chains of the branched and linear types, respectively, and that the individual antibodies recognize different oligosaccharide domains (for review see [26]).

Gastric-mucosal sheep glycoproteins which possess some oligosaccharide fractions identical to the tetrasaccharide in cow κ-caseinoglycopeptide have been shown to express the blood group I antigen [28, 29]. It is possible to suggest that in the cow the synthesis of these oligosaccharides might be regulated by the β-6 GlcNAc-transferase which allows the synthesis of branched structures and is no longer produced when the synthesis of mature milk starts.

REFERENCES

1. Fournet, B., Fiat, A. M., Alais, C. & Jollès, P. (1979) *Biochim. Biophys. Acta 576*, 339–346.
2. van Halbeek, H., Dorland, L., Vliegenthart, J. F. G., Fiat, A. M. & Jollès, P. (1980) *Biochim. Biophys. Acta 623*, 295–300.
3. Guérin, J., Alais, C., Jollès, J. & Jollès, P. (1974) *Biochim. Biophys. Acta 351*, 325–332.
4. Fiat, A. M., Jollès, J., Loucheux-Lefebvre, M. H., Alais, C. & Jollès, P. (1981) *Hoppe-Seyler's Z. Physiol. Chem. 362*, 1447–1454.
5. Fournet, B., Fiat, A. M., Montreuil, J. & Jollès, P. (1975) *Biochimie (Paris) 57*, 161–165.
6. Saito, T., Itoh, T., Adachi, S., Suzuki, T. & Usui, T. (1981) *Biochim. Biophys. Acta 678*, 257–267.

7. Saito, T., Itoh, T. & Adachi, S. (1981) *Biochim. Biophys. Acta 673*, 487–494.
8. Saito, T., Itoh, T., Adachi, S., Suzuki, T. & Usui, T. (1982) *Biochim. Biophys. Acta 719*, 309–317.
9. van Halbeek, H., Dorland, L., Vliegenthart, J. F. G., Fiat, A. M. & Jollès, P. (1981) *FEBS Lett. 133*, 45–50.
10. McKenzie, H. A. & Wake, R. G. (1961) *Biochim. Biophys. Acta 47*, 240–242.
11. Carlson, D. M. (1961) *J. Biol. Chem. 241*, 2984–2986.
12. Schultze, H. E., Schmidtberger, R. & Haupt, R. (1958) *Biochem. Z. 329*, 490–507.
13. Warren, L. (1959) *J. Biol. Chem. 234*, 1971–1975.
14. Ernst, R. R. (1966) *Adv. Magn. Res. 2*, 1–135.
15. Vliegenthart, J. F. G., Dorland, L. & van Halbeek, H. (1983) *Adv. Carbohydr. Chem. Biochem. 41*, 209–374.
16. Gooi, H. C., Uemura, K., Edwards, P. A. W., Foster, C. S., Peckering, N. & Feizi, T. (1983) *Carbohydr. Res. 120*, 293–302.
17. Uemura, K., Childs, R. A., Hanfland, P. & Feizi, T. (1983) *Biosci. Rep. 3*, 577–588.
18. Piller, F., Cartron, J. P., Maranduba, A., Veyrières, A., Leroy, Y. & Fournet, B. (1984) *J. Biol. Chem. 259*, 13385–13390.
19. Fracker, P. J. & Speck, J. C. (1978) *Biochem. Biophys. Res. Commun. 80*, 849–857.
20. van Halbeek, H., Dorland, L., Vliegenthart, J. F. G., Hull, W. E., Lamblin, G., Lhermitte, M., Boersma, A. & Roussel, P. (1982) *Eur. J. Biochem. 127*, 7–20.
21. Kanamori, M., Doi, H., Ideno, S. & Ibuki, F. (1981) *J. Nutr. Sci. Vitamol. 27*, 231–241.
22. Doi, H., Kobatake, H., Ibuki, F. & Kanamori, M. (1980) *Agric. Biol. Chem. 44*, 2605–2611.
23. Zevaco, C. & Ribadeau-Dumas, B. (1984) *Milchwissenschaft 39*, 206–210.
24. Vreeman, H. J., Visser, S., Slangen, C. J. & Van Riel, J. A. M. (1986) *Biochem. J. 240*, 87–97.
25. Slomiany, B. L., Zdebska, E. & Slomiany, A. (1984) *J. Biol. Chem. 259*, 2863–2869.
26. Feizi, T. (1981) *Immunol. Commun. 10*, 127–156.
27. Lemieux, R. U., Wong, T. C., Liao, J. & Kabat, E. A. (1984) *Mol. Immunol. 21*, 751–759.
28. Wood, E., Hounsell, E. F., Langhorne, J. & Feizi, T. (1980) *Biochem. J. 187*, 711–718.
29. Hounsell, E. F., Wood, E., Feizi, T., Fukuda, M., Powell, M. E. & Hakomori, S. I. (1981) *Carbohydr. Res. 90*, 283–307.