*Biochimica et Biophysica Acta* 911 (1987) 244–251
Elsevier

BBA 32696

# Determination of the structure of the carbohydrate chains of acid α-glucosidase from human placenta

Johanna H.G.M. Mutsaers [a], Herman Van Halbeek [a], Johannes F.G. Vliegenthart [a], Joseph M. Tager [b], Arnold J.J. Reuser [c], Marian Kroos [c] and Hans Galjaard [c]

[a] *Department of Bio-Organic Chemistry, University of Utrecht, Utrecht (The Netherlands),* [b] *Laboratory of Biochemistry, University of Amsterdam, Amsterdam (The Netherlands)* and [c] *Department of Cell Biology and Genetics, Erasmus University, Rotterdam (The Netherlands)*

(Received 30 June 1986)

Key words: Acid α-glucosidase; Carbohydrate chain; HPLC; NMR; (Human placenta)

**Acid α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) from human placenta (70 and 76 kDa) was found to contain 4 *N*-glycosidic carbohydrate chains per molecule. Sugar analysis of purified enzyme revealed the presence of mannose, *N*-acetylglucosamine and fucose at a molar ratio of 5.0:2.0:0.6. In addition, trace amounts of galactose and *N*-acetylneuraminic acid were detected. The sugar chains were liberated from the polypeptides by the hydrazinolysis procedure and subsequently fractionated by gel filtration and HPLC. Purified compounds were investigated by 500-MHz $^1$H-NMR spectroscopy. Oligomannoside-type chains of intermediate size, e.g., $Man_5GlcNAcGlcNAc$-ol and $Man_7GlcNAcGlcNAc$-ol, and *N*-type chains of smaller size e.g., $Man_{2-3}GlcNAc[Fuc]_{0-1}GlcNAc$-ol, were demonstrated to be present at a ratio of 2:3. In addition, a small amount of sialylated *N*-acetyllactosamine-type chains has been found. The possible biosynthetic route of the fucose-containing small-size chains is discussed.**

## Introduction

Acid α-glucosidase is a lysosomal enzyme that catalyses the complete hydrolysis of glycogen. Glycogenosis type II (Pompe disease) is an inherited lysosomal glycogen storage disorder caused by acid α-glucosidase deficiency; the disease may occur in a variety of phenotypes [1]. Recent studies on the biosynthesis of acid α-glucosidase in cultured fibroblasts from clinical variants have shown that some specific types of mutation may lead to alterations of the protein backbone and/or its carbohydrate side-chains [2]. In two variants, for example, phosphorylation of mannose residues, which is essential for recognition of the enzyme by the mannose 6-phosphate receptor, could not be demonstrated [2].

To define further these particular types of mutation and to elucidate their effect on the carbohydrate moiety, more detailed information on the number and structure of the oligosaccharide chains of normal acid α-glucosidase appeared to be essential. We present here the structural analysis of the carbohydrate moiety of human placenta acid α-glucosidase.

Abbreviations: HPLC, high-performance liquid chromatography; Man, mannose; Fuc, fucose; Gal, galactose; GlcNAc, *N*-acetylglucosamine; NeuAc, *N*-acetylneuraminic acid; GlcNAc-ol, *N*-acetylglucosaminitol; SDS, sodium dodecyl sulfate.

Correspondence: J.F.G. Vliegenthart, Department of Bio-Organic Chemistry, Transitorium III, P.O. Box 80.075, NL-3508 TB Utrecht, The Netherlands.

## Materials and Methods

*Isolation and purification of acid α-glucosidase from human placenta.* Acid α-glucosidase was isolated from human placenta as described before [2,3]. Briefly, the procedure involves the isolation of concanavalin A-binding glycoproteins and subsequent purification of acid α-glucosidase by affinity chromatography using Sephadex G-200 as affinity matrix.

*Gel electrophoresis.* Polyacrylamide gel electrophoresis in the presence of SDS and mercaptoethanol was carried out as described [4]. The gels were stained for protein with Coomassie blue and for carbohydrate with periodic-acid/Schiff reagent [5].

*Sugar analysis.* Acid α-glucosidase (570 μg) was subjected to methanolysis (1.0 M methanolic HCl, 24 h, 85°C) followed by gas-liquid chromatography of the trimethylsilylated (re-)*N*-acetylated methyl glycosides on a capillary CPsil-5 WCOT fused silica column (0.34 mm × 25 m, Chrompack) [6].

*Hydrazinolysis procedure.* Acid α-glucosidase (30 mg) was dialysed against 1 l double-distilled water (20 h, 4°C) with one intermediate change. After lyophilization, the dialysate was thoroughly dried in vacuo over $P_2O_5$.

For hydrazinolysis the α-glucosidase was suspended in 2 ml anhydrous hydrazine and heated for 8 h at 100°C. After evaporation of hydrazine, the material was (re-)*N*-acetylated and reduced as described [7]. For reduction with $^3$H-labelled $NaBH_4$, 0.1 part of the sample was dissolved in 200 μl 0.08 M NaOH and treated with $NaBH_4$ containing 1.8 mCi $NaB^3H_4$ in 215 μl *N*,*N*-dimethylformamide. The remaining part was reduced with $NaB^2H_4$. To facilitate the detection of $NaB^2H_4$-reduced oligosaccharides, 0.1 μCi (1/40 part) of the $^3$H-labelled oligosaccharide alditols was added.

*Fractionation and purification of carbohydrate chains.* High-voltage paper electrophoresis was performed by applying the hydrazinolysate onto Whatman 3MM paper in a buffer of pyridine/acetic acid/water, 3:1:387 (v/v), pH 5.5, at 70 V/cm.

The mixture of neutral oligosaccharide alditols recovered from the paper was fractionated on Bio-Gel P-4 (2 × 100 cm, under 400 mesh; Bio-Rad). The column was eluted with double-distilled water at 55°C [8].

HPLC was performed on a 5 μm Lichrosorb-$NH_2$ column (25 × 0.46 cm i.d., Merck). Elution was carried out isocratically with acetonitrile/water at a ratio of 64:36 (v/v) (F1) or 70:30 (v/v) (F2, F3 and F4) at a flow rate of 1 ml/min [9]. As reference compounds $Man_9GlcNAcGlcNAc$-ol and $Man_6GlcNAcGlcNAc$-ol from prostaglandin endoperoxide synthase [10], $Man_5GlcNAcGlcNAc$-ol from ribonuclease B (Gerwig, van Halbeek, Vliegenthart, unpublished results) and $Man_3GlcNAcGlcNAc$-ol from hen ovomucoid [11] were used.

*500-MHz $^1$H-NMR spectroscopy.* Deuterium-exchanged oligosaccharide alditols were obtained by three-fold lyophilization of their $^2H_2O$ solutions. Finally the samples were dissolved in 0.4 ml $^2H_2O$ (99.96 atom% $^2$H, Aldrich). 500-MHz $^1$H-NMR spectra were recorded on a Bruker WM 500 instrument (SON hf-NMR facility, Department of Biophysical Chemistry, Nijmegen Univeristy, The Netherlands) operating in the pulsed Fourier-transform mode at a probe temperature of 27°C or 33°C. Chemical shifts (δ) are expressed at 27°C or 33°C in ppm downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate, but were actually measured by reference to internal acetone (δ 2.225 ppm) with an accuracy of 0.002 ppm [12].

## Results

SDS-polyacrylamide gel electrophoresis showed acid α-glucosidase to consist of two major polypeptides of 70 and 76 kDa (fig. 1). The molecular forms occur in the approximate ratio of 2 to 3, respectively. A minor component, 95 kDa, is present as an intermediate in the biosynthesis of acid α-glucosidase (see Refs. 2 and 3; not visible in Fig. 1). Both major molecular forms contain carbohydrate as judged by staining with periodic-acid/Schiff reagent (Fig. 1).

The total carbohydrate content of the acid α-glucosidase preparation was 7%. Sugar analysis revealed the presence of Man, GlcNAc and Fuc in the molar ratio of 5.0:2.0:0.6 (Man taken as 5; GlcNAc corrected for the amount of Asn-linked

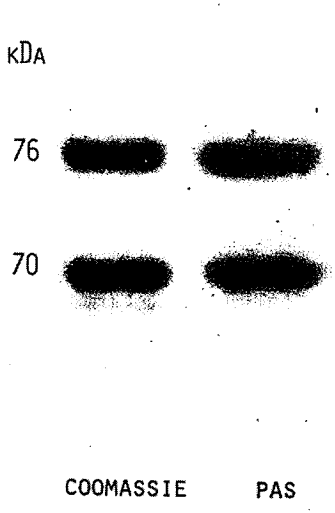


Fig. 1. SDS-polyacrylamide gel electrophoresis of placental acid α-glucosidase stained with Coomassie blue and with periodic acid/Schiff reagent (PAS). When more sample is loaded onto the gel a band at 95 kDa becomes visible.

GlcNAc that is not released under the applied conditions of methanolysis). In addition, traces of Gal, Glc and NeuAc were found. This composition points to the presence of *N*-glycosidically linked carbohydrate chains.

Hydrazinolysis was applied to release the carbohydrate moiety from the polypeptide backbone. High-voltage paper electrophoresis of the (re-)*N*-acetylated, reduced hydrazinolysate showed that over 97% consisted of neutral material. Two acidic fractions, A1 and A2, were present, amounting to 1.5 and 0.8% of total carbohydrate, respectively. The latter were directly subjected to $^1$H-NMR spectroscopy.

The neutral oligosaccharide alditols were fractionated on Bio-Gel P-4. The elution profile is shown in Fig. 2. Fractions F1 to F4 were pooled as indicated. Their elution positions correspond to those of glucose oligomers of $10.5 \pm 1.0$, $8.5 \pm 1.0$, $6.7 \pm 0.7$ and $5.5 \pm 0.5$ residues, respectively. These fractions were then subjected to 500-MHz $^1$H-NMR spectroscopy. The relevant NMR data have been compiled in Table I.

F1 was shown to consist of a mixture of oligomannoside-type alditols. The reduced *N,N'*-diacetylchitobiose unit is characterized by the chemical shifts of H-2 and NAc of GlcNAc-1-ol and of H-1 and NAc of GlcNAc-2 (see Table I; cf.

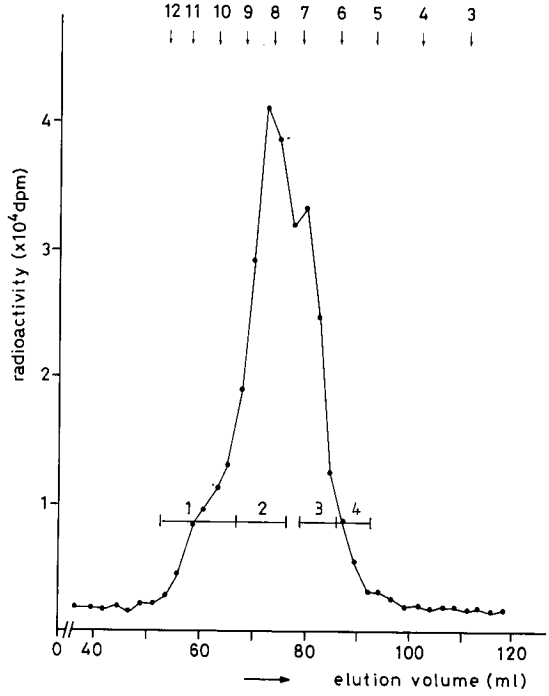


Fig. 2. Bio-Gel P-4 elution profile of the (re-)*N*-acetylated reduced hydrazinolysate obtained from human placenta acid α-glucosidase. Fractions F1 to F4 were pooled as indicated. The arrows at the top indicate the elution positions of glucose oligomers present in a dextran partial hydrolysate. The numbers above the arrows indicate the number of glucose units.

Ref. 12). In addition, all compounds of F1 share the $Man_5$-moiety consisting of Man-3, -4, -4', -A and -B. In contrast, Man-C and Man-$D_3$ are only partly present. The latter is reflected in the $^1$H-NMR spectrum of F1 by the presence of two signals for H-1 of Man-4, at δ 5.098 (representing a terminal Man-4, cf. compound 62 in Ref. 12) [13] and at δ 5.348 (for a Man-4 substituted by Man-C) [11]. From the relative intensities of these signals (1 : 1) it was estimated that Man-C is present in 50% of the molecules. For H-1 of Man-B also two signals are observed, namely at δ 4.907, (pointing to Man-B in terminal position), and at δ 5.142 (for Man-B substituted with Man-$D_3$) [10]. Like Man-C, Man-$D_3$ was estimated to be present in about 50% of the constituent molecules of F1. From the NMR spectrum it was not possible to determine whether F1 consists of a mixture of $Man_6$ compounds and/or of a $Man_7$ and a $Man_5$ compound. HPLC analysis of F1, however, showed

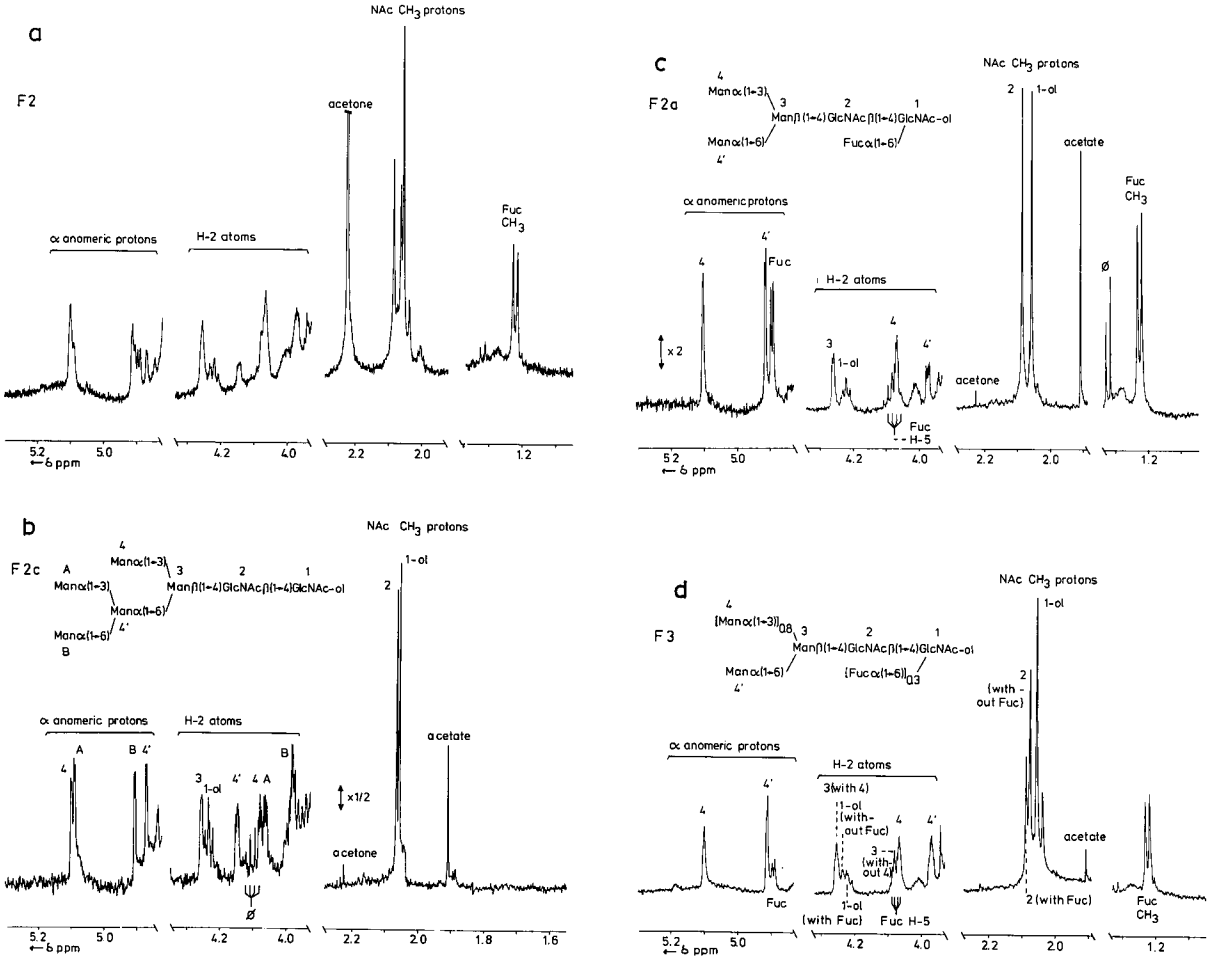


Fig. 3. Resolution-enhanced structural-reporter-group regions of the 500-MHz $^1$H-NMR spectra of the major oligosaccharide alditols derived from acid α-glucosidase in $^2H_2O$ at 27°C. (a) fraction F2, (b) fraction F2c, (c) fraction F2a, (d) fraction F3.

the presence of only two peaks. By interpolation of the retention times of the reference compounds $Man_9GlcNAcGlcNAc$-ol, $Man_6GlcNAcGlcNAc$-ol and $Man_5GlcNAcGlcNAc$-ol, these could be assigned to the $Man_7$ and the $Man_5$ compound. In conclusion, the structures of the F1 components are as shown in Scheme I (F1a and F1b).

The $^1$H-NMR spectrum of Bio-Gel P-4 fraction F2 (see Fig. 3a), like that of F1, shows characteristic features of oligomannoside-type alditols. In addition, the occurrence of an α-linked fucose residue is recognised from structural-reporter group signals at δ 4.893 (H-1), δ 4.076 (H-5) and δ 1.225 ($CH_3$) [12]. The intensity ratios of various anomeric-proton signals being non-integers (Fig. 3a) suggested that F2 consists of a mixture of compounds. The sites of heterogeneity are the Man-A, -B, -C residues and the Fuc residue. However, the NMR spectrum of the mixture F2 was too complex to determine the structures of the various components without further separation. Application of HPLC resulted in three subfractions, designated F2a, F2b and F2c. The 500-MHz $^1$H-NMR spectra recorded of F2a and F2c are shown in Fig. 3. The relevant NMR data have been included in Table I.

The NMR spectrum of F2c (Fig. 3b) shows the presence of a single oligosaccharide alditol that

possesses 5 Man residues, and ends in the reduced *N*,*N*′-diacetylchitobiose unit (see Table II, cf. F1b) [10]. Comparison of the chemical shifts of the H-1 signals of the Man residues of F2c with those of $Man_5GlcNAc_2Asn$ [13,14] shows that they are identical. Therefore, F2c has the same structure as F1b, see Scheme I.

The NMR spectrum of F2b shows close similarity with that of F2c. The change in chemical shift of one of the *N*-acetyl signals, from δ 2.055 (GlcNAc-1-ol) to 2.038, the absence of the H2 signal of a reduced GlcNAc at $4.2 < \delta < 4.3$ and the small shift effect on the H-1 signal of Man-A in comparison with F2c suggest that F2b contains a modified GlcNAc-1 residue at the reducing end.

The spectrum of F2a (Fig. 3c) shows a pure compound. This oligosaccharide alditol appeared to contain the Fuc residue. The sets of H-1/H-2 chemical shifts for Man-4 (δ 5.104/4.064) and Man-4′ (δ 4.915/3.971) are typical of the terminal nonreducing position of these residues [11,12]. The Fuc residue present in F2a is α(1-6)-linked to GlcNAc-1-ol. This can be deduced from the chemical shifts of the Fuc structural-reporter groups (δ H-1 4.893, δ H-5 4.076 and δ $CH_3$ 1.224). The latter are in accord with those found for the biantennary, *N*-acetyllactosamine-type oligosaccharide alditol ending in Manβ(1–4)GlcNAcβ(1–4)[Fucα(1–6)]GlcNAc-ol derived from IgM (ZAJ) [14], and also with those reported for α-hemocyanin Manα(1–6)[Xyl1β(1–2)]Manα(1–3)Manβ(1–4)GlcNAcβ(1–4)[Fucα(1–6)]GlcNAc-ol from *Helix pomatia* [15]. The effects on the chemical shifts of neighbouring structural-reporter groups, due to the apparent attachment of Fuc to GlcNAc-1-ol (compare F2a to F2c in Table I and to compound 1 in Ref. 11), are restricted to H-1 and NAc of GlcNAc-2 and H-2 of GlcNAc-1-ol (cf. Ref. 15). Therefore, F2a has the structure shown in Scheme I.

Bio-Gel P-4 fraction F3 contains a mixture of rather small-size compounds. An analytical run on HPLC showed that it consisted mainly of $Man_3GlcNAcGlcNAc$-ol. The $^1$H-NMR spectrum (see Fig. 3d) shows that the element Manα(1–6)Manβ(1–4)GlcNAcβ(1–4)GlcNAc-ol is present in all components of F3. In addition, Man-4 was found to occur in about 80% of the molecules in F3. This was derived from the presence and relative intensity of the H-1 and H-2 signals of Man-4 at δ 5.102 and 4.066, respectively, in conjunction with the H-2 signal of 3,6-disubstituted Man-3 at δ 4.254. The relatively low Man-3 H-2 signal at δ 4.080 (relative intensity 20%) indicated that F3 also contained a component without Man-4. Furthermore, a Fuc residue α(1–6)-linked to GlcNAc-1-ol (compare F2a, Table I) was found in about 30% of the molecules in F3. Thus the structure of the F3 compounds can be summarized as shown in Scheme I.

| | | Relative amount in % |
|---|---|---|
| F1a | Manα(1–2)Manα(1–3)<br>Manα(1–3) ⟩Manβ(1–4)GlcNAcβ(1–4)GlcNAc-ol<br>⟩Manα(1–6)<br>Manα(1–2)Manα(1–6) | 10 |
| F1b, F2c | Manα(1–3)<br>Manα(1–3) ⟩Manβ(1–4)GlcNAcβ(1–4)GlcNAc-ol<br>⟩Manα(1–6)<br>Manα(1–6) | 27 |
| F2a | Manα(1–3)<br>⟩Manβ(1–4)GlcNAcβ(1–4)GlcNAc-ol<br>Manα(1–6) Fucα(1–6)⟋ | 30 |
| F3 | $[Man\alpha(1–3)]_{0.8}$<br>⟩Manβ(1–4)GlcNAcβ(1–4)GlcNAc-ol<br>Manα(1–6) $[Fuc\alpha(1–6)]_{0.3}$⟋ | 20 |
| F4 | $[Man\alpha(1–3)]_{0.2}$<br>⟩Manβ(1–4)GlcNAcβ(1–4)GlcNAc-ol<br>Manα(1–6) $[Fuc\alpha(1–6)]_{0.1}$⟋ | 8 |

Scheme I. Structures and estimated relative abundances of the neutral oligosaccharide alditols derived from acid α-glucosidase.

Fraction F4 consists mainly (≈ 70%) of the tetrasaccharide Man$\alpha$(1–6)Man$\beta$(1–4)GlcNAc-$\beta$(1–4)GlcNAc-ol. The set of H-1 and H-2 signals for Man-3 at $\delta$ 4.776 and 4.083, and for Man-4′ at $\delta$ 4.917 and 3.973, point to this structure (cf. compound 3 in Ref. 12). In addition, Man-4 is present in about 20% of F4 molecules; for the latter the Man-4 H-1 and H-2 signals in the NMR spectrum are observed at $\delta$ 5.107 and 4.073, respectively, while H-2 of Man-3 resonates at $\delta$ 4.251. Furthermore, in about 10% of the F4 material, Fuc $\alpha$(1–6)-linked to GlcNAc-ol is present. Thus, the structures of F4 can be summarized as shown in Scheme I.

The structures of the acidic fractions A1 and A2 were also investigated by $^1$H-NMR spectroscopy. Fraction A1 appeared to be a mixture of oligomannoside-type (50%) and *N*-acetyllactosamine-type (50%) structures. The oligomannoside-type structures are mainly $Man_5$ and $Man_7$ compounds, containing the same residues as given for F2c and F1. The *N*-acetyllactosamine-type chains were found to be of the biantennary type, mono-$\alpha$(2–6)-sialylated at the $\alpha$(1–3) branch ($\delta$ H-1

TABLE I

$^1$H-NMR CHEMICAL SHIFTS OF STRUCTURAL-REPORTER GROUPS OF THE CONSTITUENT MONOSACCHARIDES OF THE HYDRAZINOLYSATE COMPONENTS DERIVED FROM HUMAN PLACENTA ACID $\alpha$-GLUCOSIDASE

Chemical shifts are expressed in ppm downfield from internal DSS (sodium 4,4-dimethyl-4-silapentane-1-sulfonate) at 27°C, except for F4 (33°C), but were actually measured by reference to internal acetone ($\delta$ 2.225) with an accuracy of 0.002 ppm. For coding of residues and complete structures see Scheme I.

| Reporter group | Chemical shift in | | | | | | |
|---|---|---|---|---|---|---|---|
| | F1a | F1b | F2c | F2b | F2a | F3 | F4 |
| | C–4<br>A\ >3–2–1–ol<br>4′<br>$D_3$–B/ | 4<br>A\ >3–2–1–ol<br>4′<br>B/ | | 4<br>A\ >3–2–1–?<br>4′<br>B/ | 4<br>>3–2–1–ol<br>4′ F/ | $(4)_{0.8}$<br>>3–2–1–ol<br>4′ $(F)_{0.3}$/ | $(4)_{0.2}$<br>>3–2–1–ol<br>4′ $(F)_{0.1}$/ |
| H-1 2 | 4.627 | 4.627 | 4.629 | – | 4.72 | 4.72 [a]/4.635 [b] | n.d. [a]/4.641 [b] |
| 4 | 5.348 | 5.098 | 5.100 | 5.094 | 5.104 | 5.102 [c] | 5.107 [c] |
| | 4.868 | 4.868 | 4.871 | 4.869 | 4.915 | 4.912 | 4.917 |
| A | 5.089 | 5.089 | 5.090 | 5.094 | – | – | – |
| B | 5.142 | 4.907 | 4.906 | 4.904 | – | – | – |
| C | 5.052 | – | – | – | – | – | – |
| $D_3$ | 5.039 | – | – | – | – | – | – |
| Fuc | – | – | – | – | 4.893 | 4.893 [a] | 4.89 [a] |
| H-2 1-ol | 4.232 | 4.232 | 4.234 | – | 4.219 | 4.225 [a]/4.235 [b] | 4.226 [a]/4.236 [b] |
| 3 | 4.232 | 4.253 | 4.256 | 4.251 | 4.259 | 4.254 [c]/4.080 [d] | 4.251 [c]/4.083 [d] |
| 4 | 4.114 | 4.066 | 4.076 | 4.069 | 4.064 | 4.066 [c] | 4.073 [c] |
| 4′ | 4.145 | 4.145 | 4.147 | 4.143 | 3.971 | 3.968 | 3.973 |
| A | 4.066 | 4.066 | 4.062 | 4.069 | – | – | – |
| B | 4.023 | 3.98 | 3.985 | 3.985 | – | – | – |
| C | 4.066 | – | – | – | – | – | – |
| $D_3$ | 4.066 | – | – | – | – | – | – |
| H-5 Fuc | – | – | – | – | 4.076 | 4.075 [a] | 4.073 [a] |
| $CH_3$ Fuc | – | – | – | – | 1.224 | 1.223 [a] | 1.228 [a] |
| NAc 1-ol | 2.055 | 2.055 | 2.055 | 2.038 | 2.055 | 2.056 | 2.058 |
| 2 | 2.063 | 2.063 | 2.064 | 2.064 | 2.085 | 2.086 [a]/2.075 [b] | 2.085 [a]/2.080 [b] |

[a] When GlcNAc-ol is fucosylated at C-6.
[b] When GlcNAc-ol is nonfucosylated.
[c] When Man-4 is present.
[d] When Man-4 is absent.

Man-4 5.137, δ H-1 Man-4′ 4.910, δ H-2 Man-3 4.253, δ H-2 Man-4 4.196, δ H-2 Man-4′ 4.12, δ H-3ax NeuAc 1.719, and δ H-3eq NeuAc 2.675) [12]. Part (50%) of the oligosaccharide material in this acidic fraction A1 was present in the form of glycopeptides. This is evident from the occurrence of an *N*-acetyl signal in the NMR spectrum at δ 2.007, typical for GlcNAc-1 linked to Asn [12]. The intensity ratio of this signal and that of NeuAc (δ 2.031) was estimated to be 1 : 1. The remaining portion (50%) of the material of A1 was present in the form of reduced oligosaccharides.

Fraction A2 appeared to consist mainly of bi-antennary structures of the *N*-acetyllactosamine type, but α(2–6)-sialylated in both branches (δ H-1 Man-4 5.133, δ H-1 Man-4′ 4.948, δ H-3ax NeuAc 1.716 and δ H-3eq NeuAc 2.667) [12].

## Discussion

Among the carbohydrate chains of glycoconjugates that serve as recognition markers, those of lysosomal enzymes are best-characterized. The carbohydrate moiety of newly synthesized lysosomal enzymes is crucial for their targeting to the lysosomes, as shown for fibroblasts and for certain other cell types [16]. Two receptors for such chains containing mannose 6-phosphate have been identified [17].

The carbohydrate structures of only a few lysosomal enzymes have been studied in detail. For the enzymes studied so far, there seems to be little tissue and species specificity. Comparing different lysosomal enzymes from the same tissue/species, their carbohydrates may vary from one to another. Soluble glycoproteins, such as β-glucuronidase from rat liver [18] and human spleen [19,20], cathepsin D from porcine spleen [21,22] and cathepsin H from rat liver [23], contain predominantly $Man_{5-8}GlcNAc_2$ oligomannoside type of chains, some of which may be phosphorylated. Cathepsin B from rat liver and porcine spleen contains smaller-size ($Man_{2-3}GlcNAc_2$) *N*-glycosidic chains [23,24]. For cathepsin B from rat liver and porcine spleen [23,24] and β-glucuronidase from human spleen [20] these smaller-size *N*-glycosidic chains may be fucosylated ($Man_{2-3}GlcNAc$[Fuc]GlcNAc). Oligomannoside-type chains that are fucosylated at GlcNAc-1, ($Man_5GlcNAc$-[Fuc]GlcNAc) are present as minor compounds in cathepsin D from porcine spleen [22] and β-glucuronidase from human spleen [20]. In the latter enzyme also a small amount (5%) of *N*-acetyllactosamine type of chains has been reported to be present [20]. In contrast, the membrane-bound enzyme β-glucocerebrosidase from human placenta, which is independent of the mannose 6-phosphate receptor for its translocation to the lysosomes [25,26], contains predominantly (80%) sialylated bi- and tri-antennary *N*-acetyllactosamine type of structures [27].

In this study we have found that the soluble enzyme acid α-glucosidase from human placenta contains predominantly oligomannoside type of chains (see Scheme I), which fits the general picture outlined above. Its neutral carbohydrates can be divided into two classes: (a) chains of intermediate size, such as $Man_5GlcNAc_2$ and $Man_7GlcNAc_2$ (F1, F2c), and (b) smaller-size chains such as $Man_2GlcNAc_2$ and $Man_3GlcNAc_2$ (F3, F4). However, α-glucosidase is exceptional in its relatively high amount of fucosylated chains. The smaller chains (F2a, F3, F4) may be extended by a Fuc residue α(1–6)-linked to GlcNAc-ol. Finally, a tiny amount of sialylated bi-antennary *N*-acetyllactosamine type of chains was found. The finding of oligomannoside-type chains in the acidic fraction A1 of the hydrazinolysate is probably best explained by their linkage to residual peptides, not cleaved by hydrazine. Since the human placenta enzyme is not a high-uptake form [3], phosphorylation is not a very probable explanation for the acidic nature of the compounds in A1.

On the basis of the molecular mass of the purified enzyme (average 73.5 kDa), its carbohydrate content (7% w/w) and the structures of the carbohydrates (Scheme I; average 1300 Da), it can be estimated that there are 4 glycosylation sites per polypeptide chain.

Interestingly, we observed Fuc α(1–6)-linked to GlcNAc-ol in most of the smaller-size oligosaccharide alditols obtained from α-glucosidase. According to concepts of biosynthesis of *N*-linked carbohydrates with respect to fucosylation at the Asn-linked GlcNAc residue, the fucosyltransferase involved cannot act until at least one of the β(1–2)-linked GlcNAc residues has been attached to the $Man_{3(or\ 5)}GlcNAc_2Asn$ core structure [28].

Evidence for this concept stems from in vitro experiments, using an $\alpha(1-6)$-fucosyltransferase purified from porcine liver. It remains to be established how the presence of a fucose residue in the $\alpha$-glucosidase short carbohydrate chains can be explained. Obviously, if a GlcNAc$\beta(1-2)$ were attached to Man$\alpha(1-3)$ before fucosylation, it must have disappeared afterwards. Since it is known that human placenta is a rich source of $\beta$-hexosaminidase [29] it could be that this GlcNAc residue has been removed during the work-up procedure for acid $\alpha$-glucosidase. Another possibility is that in human tissues the fucosyltransferase has a substrate specificity different from that of the porcine enzyme [28], not requiring the presence of the branch GlcNAc in $\beta(1-2)$ linkage.

## Acknowledgements

This investigation was supported by the Netherlands Foundation for Chemical Research (SON/ZWO), the Netherlands Foundation for Fundamental Medical Research (FUNGO/ZWO), the Netherlands Foundation for Cancer Research (KWF, Grant UUKC 83-13) and the Rotterdam Foundation of Clinical Genetics. We thank T.B.M. Hakvoort (Amsterdam) for the assistance in enzyme purification and G.J. Gerwig (Utrecht) for sugar analyses.

## References

1 Hers, H.G. and De Barsy, T. (1973) in Lysosomes and Lysosomal Storage Diseases (Hers, H.G. and Van Hoof, F., eds.), pp. 197–216, Academic Press, New York
2 Reuser, A.J.J., Kroos, M., Oude Elferink, R.P.J. and Tager, J.M. (1985) J. Biol. Chem. 260, 8336–8341
3 Reuser, A.J.J., Kroos, M., Ponne, N.J., Wolterman, R.A., Loonen, M.C.B., Busch, H.F.M., Visser, W.J. and Bolhuis, P.A. (1984) Exp. Cell Res. 155, 178–189
4 Laemmli, U.K. (1970) Nature 227, 680–685
5 Fairbanks, G., Steck, T.L. and Wallach, D.F.G. (1971) Biochemistry 10, 2606–2617
6 Kamerling, J.P. and Vliegenthart, J.F.G. (1982) Cell Biol. Monogr. 10, 95–125
7 Takasaki, S., Mizuochi, T. and Kobata, A. (1982) Methods Enzymol. 83, 263–268
8 Yamashita, K., Mizuochi, T. and Kobata, A. (1982) Methods Enzymol. 83, 105–127
9 Boersma, A., Lhermitte, M., Lamblin, G. and Degand, P. (1981) Carbohydr. Res. 115, 175–181
10 Mutsaers, J.H.G.M., Van Halbeek, H., Kamerling, J.P. and Vliegenthart, J.F.G. (1985) Eur. J. Biochem. 147, 569–574
11 Paz-Parente, J., Strecker, G., Leroy, Y., Montreuil, J., Fournet, B., Van Halbeek, H., Dorland, L. and Vliegenthart, J.F.G. (1983) FEBS Lett. 152, 145–152
12 Vliegenthart, J.F.G., Dorland, L. and Van Halbeek, H. (1983) Adv. Carbohydr. Chem. Biochem. 41, 209–374
13 Van Halbeek, H., Dorland, L., Veldink, G.A., Vliegenthart, J.F.G., Michalski, J.-C., Montreuil, J., Strecker, G. and Hull, W.E. (1980) FEBS Lett. 121, 65–70
14 Cahour, A., Debeire, A., Hartmann, L., Montreuil, J., Van Halbeek, H. and Vliegenthart, J.F.G. (1984) FEBS Lett. 170, 343–349
15 Van Kuik, J.A., Van Halbeek, H., Kamerling, J.P. and Vliegenthart, J.F.G. (1985) J. Biol. Chem. 260, 13984–13988
16 Callahan, J.W. and Lowden, J.A. (1981) Lysosomes and Lysosomal Storage Diseases, Raven, New York
17 Hoflack, B. and Kornfeld, S. (1985) Proc. Natl. Acad. Sci. USA 82, 4428–4432
18 Mizuochi, T., Nishimura, Y., Kato, K. and Kobata, A. (1981) Arch. Biochem. Biophys. 209, 298–303
19 Natowicz, M., Baenziger, J.U. and Sly, W.S. (1982) J. Biol. Chem. 257, 4412–4420
20 Howard, D.R., Natowicz, M. and Baenziger, J.U. (1982) J. Biol. Chem. 257, 10861–10868
21 Nakao, Y., Kozutsumi, Y., Kawasaki, T., Yamashina, J., Van Halbeek, H. and Vliegenthart, J.F.G. (1984) Arch. Biochem. Biophys. 229, 43–54
22 Takahashi, T., Schmidt, P.G. and Tang, J. (1983) J. Biol. Chem. 258, 2819–2830
23 Taniguchi, T., Mizuochi, T., Towatari, T., Katunuma, N. and Kobata, A. (1985) J. Biochem. (Tokyo) 97, 973–976
24 Takahashi, T., Schmidt, P.G. and Tang, J. (1984) J. Biol. Chem. 259, 6059–6062
25 Van Dongen, J.M., Willemsen, R., Ginns, E.I., Sips, H.J., Tager, J.M., Barranger, J.A. and Reuser, A.J.J. (1985) Eur. J. Cell Biol. 39, 179–189
26 Murray, G.J., Jonsson, L.V., Sorrell, S.H., Ginns, E.I., Tager, J.M., Schram, A.W. and Barranger, J.H. (1985) Abstr. 13th International Congress of Biochemistry, p. 733, Elsevier, Amsterdam
27 Takasaki, S., Murray, G.J., Furbish, F.S., Brady, R.O., Barranger, J.A. and Kobata, A. (1984) J. Biol. Chem. 259, 10112–10117
28 Schachter, H. (1984) Clin. Biochem. 17, 3–14
29 Hasilik, A. and Neufeld, E. (1980) J. Biol. Chem. 255, 4937–4945