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Determination of the structure of the carbohydrate chains of prostaglandin endoperoxide synthase from sheep

Johanna H. G. M. MUTSAERS, Herman VAN HALBEEK, Johannis P. KAMERLING and Johannes F. G. VLIEGENTHART
Department of Bio-Organic Chemistry, State University of Utrecht

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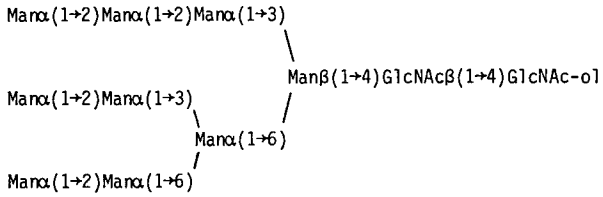
Prostaglandin endoperoxide synthase was isolated from sheep seminal vesicles. Sugar analysis of the glycoprotein revealed the presence of mannose and *N*-acetylglucosamine only. The carbohydrate moiety was released from the polypeptide backbone by hydrazinolysis. After re-*N*-acetylation and reduction, the resulting mixture of oligosaccharide-alditols was fractionated on Bio-Gel P-4 and their structures were investigated by 500-MHz ^{1}H-NMR spectroscopy. The carbohydrate chains turned out to be of the oligomannoside type containing six to nine mannose residues. The largest and most abundant compound was established to be:



For the smaller structures heterogeneity occurs with respect to the outer α(1 → 2)-linked mannose residues. Furthermore, a small amount of $Man_6GlcNAc$-ol (artefact of the hydrazinolysis procedure) was detected by ^{1}H-NMR spectroscopy and fast atom bombardment mass spectrometry.

Prostaglandin endoperoxide synthase, also known as fatty-acid cyclo-oxygenase, catalyzes the conversion of arachidonic acid into prostaglandin G_2 and the subsequent reduction of the latter to prostaglandin H_2. It contains heme and has a molecular mass of 72 kDa. The enzyme is bound to the membrane of the endoplasmic reticulum [1, 2]. Its purification from sheep and bovine seminal vesicular glands has been reported [1, 3, 4]. The sheep enzyme was established to be a glycoprotein containing 3.5% carbohydrate with D-mannose and *N*-acetyl-D-glucosamine as the only sugar constituents [1].

To gain insight into the possible role of the carbohydrate moiety in the biological functioning of the synthase, knowledge of its primary structure is prerequisite. Here, we describe the analysis of these chains employing the combination of the hydrazinolysis-procedure and 500-MHz ^{1}H-NMR spectroscopy.

MATERIALS AND METHODS

Isolation and purification of prostaglandin endoperoxide synthase

Sheep seminal vesicular glands were obtained at a local slaughterhouse, immediately frozen and stored at −20 °C. The isolation and purification of the enzyme were carried out at a temperature between 0 and 4 °C. The microsomes were isolated from vesicular glands (250 g) by differential centrifugation, followed by solubilization of the enzyme from the microsomes with Tween-20, as described [1]. The enzyme preparation (175 ml) was dialyzed for 24 h against 2 l 0.025 M Tris/HCl buffer, pH 8, containing 0.1 M EDTA and 0.1% Tween-20, with three intermediate changes. The dialyzate was subsequently applied to a DEAE-Sephadex A-50 (Pharmacia) column (2.5 × 40 cm) equilibrated with the same buffer. After washing the column with 250 ml buffer, the enzyme was eluted with a linear gradient of 0–0.4 M NaCl (800 ml). The peroxidase-active fractions were pooled and concentrated on a Diaflow XM-50 ultrafilter (Amicon). The concentrate was applied to an Ultrogel AcA-34 (LKB) column (5 × 70 cm) and eluted with a 0.05 M Tris/HCl buffer, pH 8, containing 0.1 M EDTA, 0.01% NaN_3 and 0.1% Tween-20. The peroxidase-active fractions were pooled and dialyzed for 24 h against 3 l water with three intermediate changes. The dialyzate was subsequently lyophilized and dried *in vacuo* over P_2O_5. The purity was checked by SDS-polyacrylamide gel electrophoresis according to [5].

Hydrazinolysis procedure and fractionation of carbohydrate chains

Thoroughly dried synthase (80 mg) was suspended in 1 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 [6]. For the reduction with ^{3}H-labelled $NaBH_4$ one seventh of the sample was dissolved in 200 µl

Abbreviations. EndoH, endo-β-*N*-acetyl-D-glucosaminidase H GlcNAc-ol, *N*-acetyl-D-glucosaminitol; SDS, sodium-dodecylsulfate; FAB, fast atom bombardment; NMR, nuclear magnetic resonance.

Enzymes. Endo-β-*N*-acetyl-D-glucosaminidase (EC 3.2.1.96); prostaglandin endoperoxide synthase (EC 1.14.99.1).

0.08 M NaOH and treated with $NaBH_4$ containing 1.5 mCi NaB^3H_4 in 185 µl *N,N*-dimethylformamide. The remaining part was reduced with NaB^2H_4. To facilitate the detection of oligosaccharide-alditols-1-^{2}H after the fractionation of the mixture on Bio-Gel P-4 (under 400 mesh; Bio-Rad, 2 × 100 cm) [7], 0.24 µCi (one tenth) of the ^{3}H-labelled oligosaccharide-alditols were added.

Enzyme assay

Peroxidase activity was determined by monitoring the absorbance at 610 nm of the product-formation of synthase-catalyzed oxidation of *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine in the presence of hydrogen peroxide [1].

*Treatment with endo-β-*N*-acetylglucosaminidase H*

Digestion of 20 µg purified synthase was performed with 0.5–1.0 mU endoH from *Streptomyces plicatus* (Miles) in 50 µl 10 mM sodium acetate, pH 5.5, in the presence of 0.2% SDS for 16 h at 37 °C. The reaction was stopped by heating for 5 min at 100 °C. The digest was analyzed by SDS gel electrophoresis on a 10% polyacrylamide gel according to [8].

As molecular-mass markers were used: phosphorylase *b* (98 kDa), bovine serum albumin (68 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa) and soybean trypsin inhibitor (21 kDa).

Sugar analysis

Because of interference of Tween-20 in the sugar analysis of glycoproteins by the methanolysis procedure, Tween-20 was replaced by SDS as follows. Samples containing 2 mg of glycoprotein were incubated with 6% SDS for 3 h at room temperature. The protein-SDS complex was separated from Tween-20 by gel-permeation chromatography on an Ultrogel AcA-34 column (1 × 100 cm) eluted with a 0.05 M Tris/HCl buffer, pH 8, containing 0.1 M NaCl and 0.1% SDS. Fractions containing protein were detected by SDS-polyacrylamide gel electrophoresis [5]. Pooled fractions were dialyzed for 24 h against 200 ml bidestilled water with four intermediate changes and subsequently lyophilized. The residues were subjected to methanolysis (1.0 M methanolic HCl, 24 h, 85 °C) followed by gas-liquid chromatography on a capillary CPsil-5 WCOT fused silica column (0.34 mm × 25 m, Chrompack) of the trimethylsilylated (re-*N*-acetylated) methyl glycosides [9].

500-MHz ^{1}H-NMR spectroscopy

Deuterium-exchanged oligosaccharide-alditols were obtained by five-fold lyophilization of the ^2H_2O solutions, finally using 99.96 mol% deuterated water (Aldrich). 500-MHz ^{1}H-NMR spectra were recorded on a Bruker WM 500 instrument (SON hf-NMR facility, Department of Biophysical Chemistry, Nijmegen University, The Netherlands) operating in the pulsed Fourier-transform mode at a probe temperature of 300 K. Resolution enhancement of the spectra was achieved by Lorentzian-to-Gaussian transformation from quadrature phase detection, followed by a complex Fourier transformation [10]. Chemical shifts (δ) were expressed 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 [11].

FAB mass spectrometry

Positive-ion mass spectra were recorded on a VG Analytical ZAB-HF reversed-geometry mass spectrometer (Institute for Physiological Chemistry, Bonn University, FRG). The sample, dispersed in glycerol, was bombarded with xenon atoms having a kinetic energy of approximately 9 keV. The sputtered ions were extracted and accelerated with a potential of 7 kV.

RESULTS AND DISCUSSION

Prostaglandin endoperoxide synthase was obtained from sheep seminal vesicles and purified to homogeneity by DEAE-Sephadex A-50 and Ultrogel AcA-34 chromatography. The enzyme behaved as a single band on SDS-polyacrylamide gel electrophoresis (see Fig. 1). Starting from 250 g vesicles, 40 mg enzyme was obtained. Sugar analysis of purified synthase revealed the presence of GlcNAc and Man in a

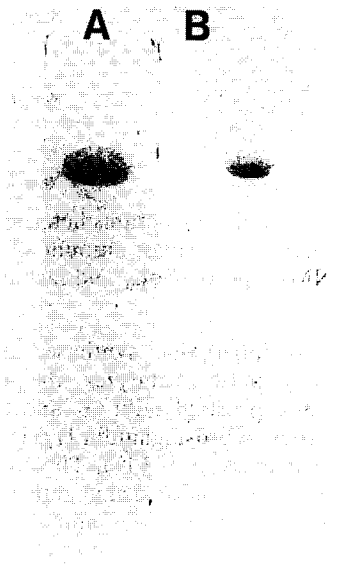


Fig. 1. *SDS-polyacrylamide gel electrophoresis of prostaglandin endoperoxide synthase.* Lane A, gel stained with periodic-acid/Schiff [12]; Lane B, gel stained with Coomassie blue G 250

Table 1. *Molar carbohydrate composition of prostaglandin endoperoxide synthase and the three oligosaccharide-alditol fractions obtained from this enzyme by the hydrazinolysis procedure*

Sample	Molar ratios		
	Man	GlcNAc	GlcNAc-ol
PGES	7.8	2.0[a]	–
F1	9.0	0.9	0.7
F2	6.2	0.8	0.7
F3	6.0	0.5[b]	0.6

[a] Corrected for the amount of Asn-linked GlcNAc that is not cleaved under the conditions used for methanolysis [13].
[b] GlcNAc in F3 is derived from a contaminant (see text).

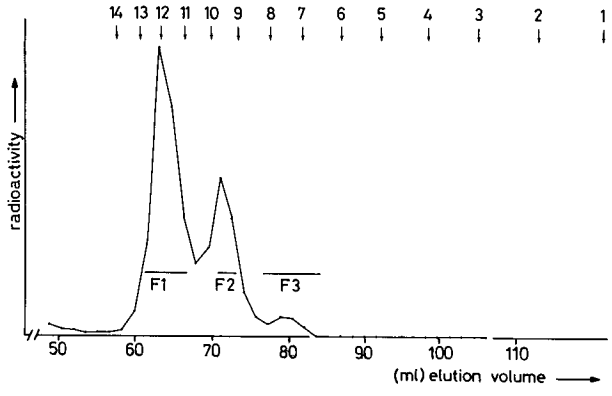


Fig. 2. *Elution profile of oligosaccharide-alditols derived from prostaglandin endoperoxide synthase on Bio-Gel P-4 (under 400 mesh).* The column was eluted with bidistilled water at 55°C. Fractions of 1.6 ml were collected at a flow rate of 20 ml/h and assayed for ^3H radioactivity. Fractions F1, F2 and F3 were pooled. The arrows at the top indicate the eluting positions of glucose oligomers derived from a dextran hydrolyzate. The numbers indicate the glucose units

molar ratio of 2.0:7.8 (see Table 1). Such a carbohydrate composition suggests the occurrence of oligomannoside type of chains, *N*-glycosidically linked to the polypeptide backbone.

Upon treatment with endoH the molecular mass of the synthase decreased from 72.5 ± 0.5 kDa to 67.5 ± 0.5 kDa, judged from SDS-polyacrylamide gel electrophoresis.

For structural analysis of the carbohydrate chains, they were released from the protein by hydrazinolysis. After re-*N*-acetylation and reduction the resulting mixture of oligosaccharide-alditols was fractionated on Bio-Gel P-4. The elution profile (Fig. 2) shows F1 and F2 as the main fractions and F3 as a minor one. The retention times of fraction F1, F2 and F3 correspond with glucose-oligomer positions of 12.1, 9.6 and 7.5 residues, respectively. These fractions were subjected to sugar analysis (see Table 1) and 500-MHz ^1H-NMR spectroscopy. The relevant NMR parameters, together with those of some reference compounds, are compiled in Table 2.

The 500-MHz ^1H-NMR spectrum of fraction F1 is shown in Fig. 3, illustrating the presence of a major and a minor

Table 2. *^1H Chemical shifts of structural-reporter-group protons of the constituent monosaccharides for the oligosaccharide-alditols derived from prostaglandin endoperoxide synthase, together with those for two reference compounds (72 and N-2b)*
Chemical shifts are given at 300 K, in ppm downfield from internal 4,4-dimethyl-4-silapentane-1-sulfonate in ^2H_2O. n. d., value could not be determined

Reporter group	Residue	Chemical shift in				
		72	F1	F2	F3	N-2b
		D_1-C-4 \ D$_2$-A 3-2-1-Asn / 4' \ D$_3$-B	D_1-C-4 \ D$_2$-A 3-2-1-ol / 4' \ D$_3$-B	C-4 \ A 3-2-1-ol / 4' \ B	C-4 \ A 3-2-ol / 4' \ B	
		ppm				
NAc of	1/1-ol	2.015	2.055	2.055	-	-
	2/2-ol	2.067	2.067	2.063	2.058	2.059
H-1 of	2/2-ol	4.610	4.630	4.628	n.d.	n.d.
	3	4.77	4.77	4.77	4.77	n.d.
	4	5.334	5.333	5.348	5.344	5.343
	4'	4.869	4.867	4.871	4.881	4.881
	A	5.404	5.400	5.092	5.137	5.133
	B	5.143	5.140	4.907	4.906	4.906
	C	5.308	5.307	5.047	5.054	5.053
	D_1	5.049	5.045	-	-	-
	D_2	5.061	5.054	-	-	-
	D_3	5.042	5.038	-	-	-
H-2 of	1-ol/2-ol	-	4.232	4.233	4.213	4.218
	3	4.228	4.232	4.236	4.245	4.243
	4	4.098	4.094	4.117	4.118	4.119
	4'	4.156	4.153	4.146	4.180	4.180
	A	4.109	4.104	4.061	4.068	4.062
	B	4.023	4.020	3.983	3.985	3.992
	C	4.109	4.104	4.068	4.068	4.067
	D_1	4.073[a]	4.071[a]	-	-	-
	D_2	4.073[a]	4.068[a]	-	-	-
	D_3	4.066[a]	4.064[a]	-	-	-

[a] Assignments may have to be interchanged.

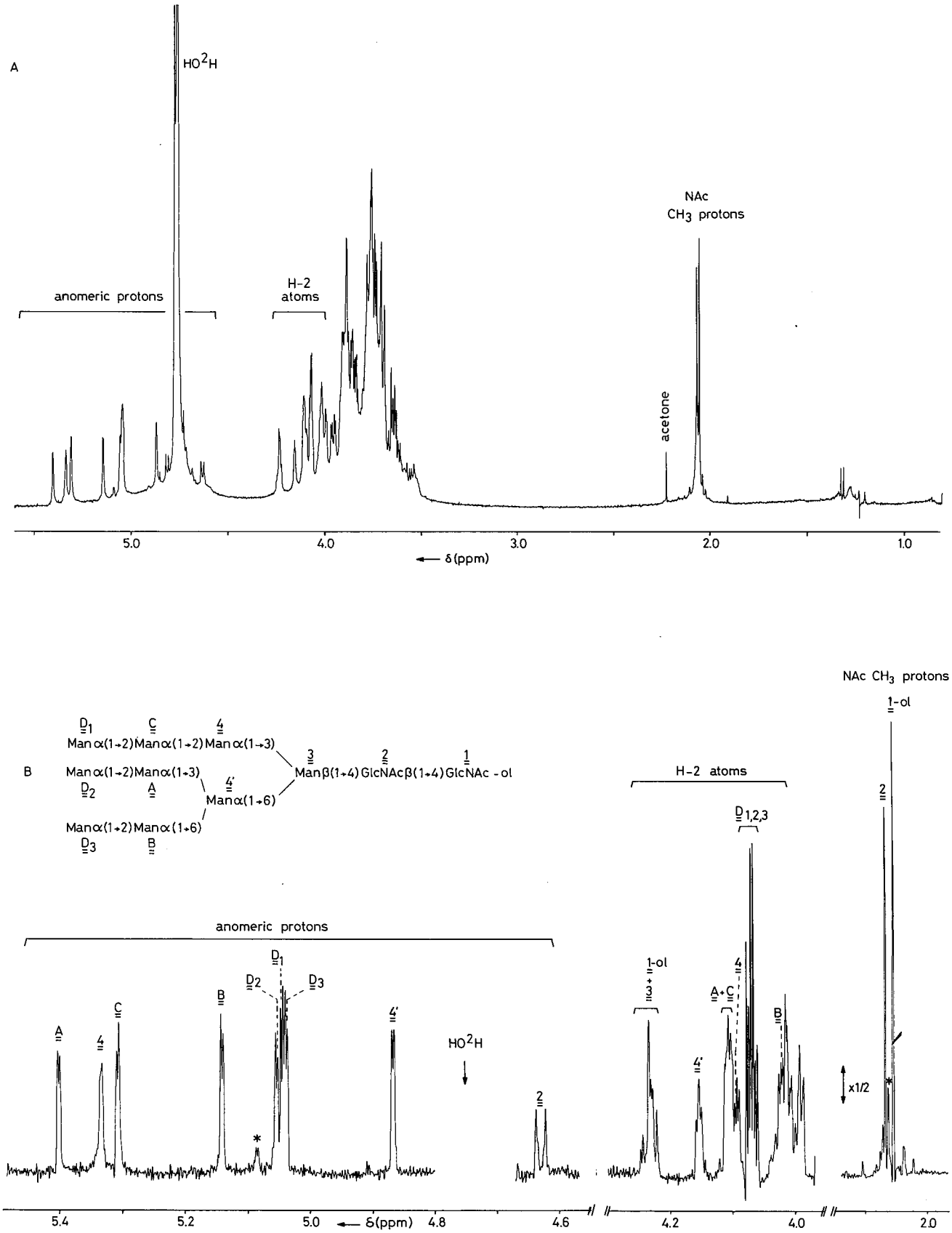
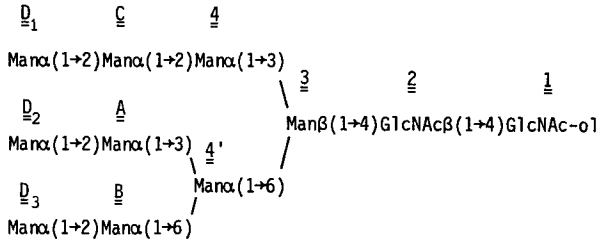


Fig. 3. *500-MHz ^{1}H-NMR spectrum of oligosaccharide-alditol fraction F1 derived from prostaglandin endoperoxide synthase, in ^2H_2O at 300 K (A) and resolution-enhanced structural-reporter-group regions of the 500-MHz ^{1}H-NMR spectrum of F1 (B).* The numbers and letters in the spectrum refer to the corresponding residues in the structure. The relative-intensity scale of the *N*-acetyl proton region differs from that for other parts of the spectrum, as indicated. Signals marked by asterisks belong to a minor (17%) component of the mixture, namely $Man_8GlcNAcGlcNAc$-ol missing Man-D_2 (see text)

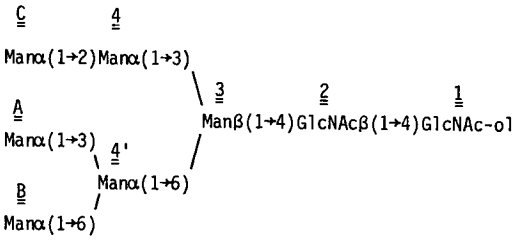
component. The spectral features of the mannose residues of the main component are essentially identical with those of the corresponding residues in $Man_9(GlcNAc)_2Asn$ from soybean agglutinin (compound 72 [11, 14]) (see Table 2). This indicates that the primary structure, with respect to the mannose residues, is the same as that of $Man_9(GlcNAc)_2Asn$.

Furthermore, the spectrum of fraction F1 shows two abundant *N*-acetyl signals. By comparison with the data of $Man_9(GlcNAc)_2Asn$, the *N*-acetyl signal at $\delta = 2.067$ ppm is assigned to GlcNAc-2. According to the sugar analysis, the second *N*-acetyl signal of $\delta = 2.055$ ppm must be attributed to GlcNAc-ol. The same chemical shift value has been found for the GlcNAc-ol residue in Manα1→3(Manα1→6)-Manβ1→4GlcNAcβ1→4GlcNAc-ol from hen ovomucoid [15]. Therefore, the structure of the main component of fraction F1 is:



The minor signals in the spectrum of fraction F1 (see Fig. 3) indicate the occurrence of $Man_8GlcNAcGlcNAc$-ol specifically missing Man-D$_2$. The low-intensity α-anomeric signal at $\delta = 5.085$ ppm is typical for H-1 of Man-A in terminal non-reducing position (compare compound 70 [11, 16]). The intensity of this signal is 17% of that of H-1 of Man-B. It should be noticed that the intensity of the H-1 signal of Man-D$_2$ is decreased in the same extent (17%) compared with the intensities of the α-anomeric signals of Man-D$_1$ and Man-D$_3$. The absence of Man-D$_2$ is also reflected in the *N*-acetyl region of the spectrum. The *N*-acetyl signal at $\delta = 2.063$ ppm is assigned to GlcNAc-2 in this minor compound because the sum of the intensities of the signals at $\delta = 2.063$ and $\delta = 2.067$ ppm equals that of the GlcNAc-ol singlet at $\delta = 2.055$ ppm. The spatial conformation of $Man_8GlcNAcGlcNAc$-ol may be held responsible for the shift alteration of the *N*-acetyl signal of GlcNAc-2 since evidence exists that the Man-A, Man-D$_2$ branch occurs in the sphere of influence of C-1 and C-2 of GlcNAc-2 [11].

In the spectrum of fraction F2 the intensity ratios of the structural-reporter-group signals are non-integers, indicating that fraction F2 consists of a mixture of compounds. Again the reduced chitobiose unit is present, as is evident from the signals at $\delta = 2.055$ ppm (GlcNAc-1-ol *N*-acetyl; unchanged in comparison with F1), $\delta = 2.063$ ppm (GlcNAc-2 *N*-acetyl), $\delta = 4.628$ ppm (GlcNAc-2 H-1) and $\delta = 4.233$ ppm (GlcNAc-1-ol H-2). The chemical shifts of the reporter groups of the main component (80% of the mixture) are compiled in Table 2. The spectrum shows five intense α-anomeric signals (the Man-3 H-1 signal is hidden under the HO^2H line). Therefore, the structure must be a $Man_6GlcNAcGlcNAc$-ol compound. The chemical shifts of the Man H-1 and H-2 signals are essentially identical with those of $Man_6(GlcNAc)_2Asn$ missing the Man-D residues (compound 63 [11, 16, 17]). The structure of the main component of fraction F2 is as follows:



The remaining 20% of fraction F2 consist of $Man_7GlcNAcGlcNAc$-ol compounds. The extension of $Man_6GlcNAcGlcNAc$-ol with Man-D$_1$ constitutes approximately 15% of the total mixture, as could be deduced from the occurrence and relative intensity (15%) of the H-1 doublet of Man-C at $\delta = 5.308$ ppm (compare compound 68 [11, 16, 17]). Another minor component (5% of the total mixture) is the extension of $Man_6GlcNAcGlcNAc$-ol with Man-D$_3$. This is evidenced by the presence and intensity (5%) of the H-1 signal of Man-B at $\delta = 5.140$ ppm. $Man_8GlcNAcGlcNAc$-ol structures are unlikely to occur in fraction F2 on the basis of the elution volume on Bio-Gel P-4.

Fraction F3 was submitted to 500-MHz ^{1}H-NMR spectroscopy and FAB mass spectrometry. The 500-MHz ^{1}H-NMR spectrum shows an oligomannoside type of structure. In the α-anomeric region of the spectrum five distinct signals are observed. The *N*-acetyl region of the spectrum shows just one abundant singlet. The structure of the main component of fraction F3 is identical to a $Man_6GlcNAc$-ol compound derived from cathepsin D which lacks specifically the Man-D residues (see Table 2, compound N-2b [18]). The signals characteristic for GlcNAc-2-ol in an oligomannoside structure are the *N*-acetyl signal at $\delta = 2.058$ ppm and the H-2 signal at $\delta = 4.213$ ppm. The most characteristic spectral feature of this compound is the exceptional position of the H-1 signal of Man-A at 5.137 ppm arising from a different spatial environment of Man-A in comparison with $Man_6GlcNAcGlcNAc$-ol.

The FAB mass spectrum of fraction F3 shows intense peaks in the high mass region at $m/z = 1197$ ($M + H$) and $m/z = 1219$ ($M + Na$). The molecular mass is in agreement with the structure derived by 500-MHz ^{1}H-NMR spectroscopy. Sugar analysis of fraction F3 showed the presence of GlcNAc in addition to Man and GlcNAc-ol. GlcNAc must stem from a contaminant, the structure of which has not been deduced so far.

In conclusion, prostaglandin endoperoxide synthase contains carbohydrate chains of the oligomannoside type. The largest and most abundant one was found to be $Man_9GlcNAcGlcNAc$-ol and the smallest one Man_6-$GlcNAcGlcNAc$-ol in which the outer α(1 → 2)-linked mannose residues (D$_1$, D$_2$ and D$_3$) are missing.

Additional compounds were $Man_8GlcNAcGlcNAc$-ol missing Man-D$_2$ and two $Man_7GlcNAcGlcNAc$-ol missing either Man-D$_1$ and -D$_2$ or Man-D$_2$ and -D$_3$. Furthermore, a small amount of $Man_6GlcNAcGlcNAc$-ol was present which is an artefact of the hydrazinolysis procedure. This phenomenon was reported earlier for oligosaccharide-alditols from hen ovomucoid [19].

The hydrazinolysis procedure, used here in combination with 500-MHz ^{1}H-NMR spectroscopy, is a convenient method for analyzing glycoproteins containing carbohydrate structures of the oligomannoside type. The reduced chitobiose unit can be readily recognized in the spectra. It is noteworthy that the chemical shift values of the structural-reporter groups

of the Man residues are close to those observed for the corresponding glycopeptides. For structures ending on GlcNAc-2-ol some significantly deviating chemical shift values are observed as is evident from comparison of the NMR data of $Man_6GlcNAc$-ol (F3), $Man_6GlcNAcGlcNAc$-ol (F2) and the glycopeptide $Man_6(GlcNAc)_2Asn$ (compound 63 [11, 16, 17]).

On the basis of the molecular mass of the enzyme before and after treatment with endoH and the carbohydrate structures elucidated, it can be estimated that there are three glycosylation sites per polypeptide chain.

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REFERENCES

1. Van der Ouderaa, F. J., Buytenhek, M., Nugteren, D. H. & Van Dorp, D. A. (1977) *Biochim. Biophys. Acta 487,* 315–331.
2. De Witt, D. L., Rollins, T. E., Day, J. S., Gaucher, J. A. & Smith, W. L. (1981) *J. Biol. Chem. 256,* 10375–10382.
3. Miyamoto, T., Ogino, N., Yamamoto, S. & Hayahishi, O. (1976) *J. Biol. Chem. 251,* 2629–2636.
4. Hemler, M., Lands, W. E. M. & Smith, W. L. (1976) *J. Biol. Chem. 251,* 5575–5579.
5. Staehelin, T. & Schreier, B. E. M. (1979) *Methods Enzymol. 60,* 135–165.
6. Takasaki, S., Mizuochi, T. & Kobata, A. (1982) *Methods Enzymol. 83,* 263–268.
7. Yamashita, K., Mizuochi, T. & Kobata, A. (1982) *Methods Enzymol. 83,* 105–127.
8. Laemmli, U. K. (1970) *Nature (Lond.) 227,* 680–685.
9. Kamerling, J. P., Gerwig, G. J., Vliegenthart, J. F. G. & Clamp, J. R. (1975) *Biochem. J. 151,* 491–495.
10. Ernst, R. R. (1966) *Adv. Magn. Res. 2,* 1–135.
11. Vliegenthart, J. F. G., Dorland, L. & Van Halbeek, H. (1983) *Adv. Carbohydr. Chem. Biochem. 41,* 209–374.
12. Fairbanks, G., Steck, T. L. & Wallach, D. F. G. (1971) *Biochemistry 10,* 2606–2617.
13. Gerwig, G. J., Kamerling, J. P. & Vliegenthart, J. F. G. (1984) *Carbohydr. Res. 129,* 149–157.
14. Dorland, L., Van Halbeek, H., Vliegenthart, J. F. G., Lis, H. & Sharon, N. (1981) *J. Biol. Chem. 256,* 7708–7711.
15. Paz-Parente, J., Strecker, G., Leroy, Y., Montreuil, J., Fournet, B., Van Halbeek, H., Dorland, L. & Vliegenthart, J. F. G. (1983) *FEBS Lett. 152,* 145–152.
16. Van Halbeek, H., Dorland, L., Vliegenthart, J. F. G., Jouanneau, J. & Bourrillon, R. (1981) *Biochem. Biophys. Res. Commun. 99,* 886–892.
17. Marti, T., Kosen, P. A., Honory, K., Franzblau, C., Schmid, K., Van Halbeek, H., Gerwig, G. J. & Vliegenthart, J. F. G. (1984) *Biochim. Biophys. Acta 799,* 305–312.
18. Nakao, Y., Kozutsumi, Y., Kawasaki, T., Yamashina, I., Van Halbeek, H. & Vliegenthart, J. F. G. (1984) *Arch. Biochem. Biophys. 229,* 43–54.
19. Egge, H., Peter-Katalinić, J., Paz-Parente, J., Strecker, G., Montreuil, J. & Fournet, B. (1983) *FEBS Lett. 156,* 357–362.

J. H. G. M. Mutsaers, H. van Halbeek, J. P. Kamerling and J. F. G. Vliegenthart,
Afdeling Bio-Organische Chemie, Organisch-Chemisch Laboratorium, Rijksuniversiteit Utrecht,
Croesestraat 79, NL-3522-AD Utrecht, The Netherlands