Volume 238, number 2, 390–394 FEB 06364 October 1988

400-MHz ^{1}H-NMR spectroscopy of fucosylated tetrasialyl oligosaccharides isolated from normal and cirrhotic α_1-acid glycoprotein

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Received 2 August 1988

The comparative study of fucosylated tetrasialyl-oligosaccharides isolated from normal and cirrhotic α_1-AGP was performed using permethylation and 400-MHz ^{1}H-NMR spectroscopy. These results clearly show the tetraantennary structure of these two oligosaccharides with hyperfucosylation for the tetrasialylated fraction from cirrhotic α_1-AGP. In the latter oligosaccharide the simultaneous presence on two antennae (7 and 7') of the sialosyl Lewis X determinant NeuAc-(α2–3) Gal(β1–4) [Fuc(α1–3)] GlcNAc has been observed. Moreover the 5 and 5' antennae were α2–6 sialylated but without fucose.

α_1-Acid glycoprotein; Fucose; Alcoholic cirrhosis; Asparagine-linked oligosaccharide; ^{1}H-NMR

1. INTRODUCTION

During human cirrhosis, hepatocellular deficiency is associated with a dramatic increase in concanavalin A-unreactive forms of α_1-acid glycoprotein [1,2], transferrin [3] and α_2-HS glycoprotein [4]. Recently [5] we have compared the glycan structure of α_1-AGP purified from cirrhotic ascitic fluid (α_1-AGPc) and normal serum (α_1-AGPn). Two major types of alterations were observed in cirrhotic α_1-AGP. First, a shift of biantennary *N*-acetyllactosamine-type oligosaccharides towards tri- and tetraantennary oligosaccharides was observed. The second major type of change was concerned with a hyperfucosylation found in all cirrhotic patients. Permethylation data of the tetrasialyl-oligosaccharide fraction indicated that all fucosyl residues were exclusively linked to external *N*-acetylglucosamine residues by an $\alpha_{1,3}$ linkage. This implies the simultaneous presence of fucosyl and sialyl residues on the same lactosamine residue, which corresponds to the sialosyl Lewis X structure [6,7].

In the present report, the tetrasialyl oligosaccharide fraction of α_1-AGPn and α_1-AGPc was reinvestigated by 400-MHz ^{1}H-NMR spectroscopy in order to establish the linkage and the location of sialyl and fucosyl residues on each antenna.

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Abbreviations: α_1-AGP, α_1-acid glycoprotein; n, normal; c, cirrhotic; OMe, *O*-methyl; GlcNAcNMeol, *N*-acetyl-*N*-methylglucosaminitol

2. MATERIALS AND METHODS

2.1. *Preparation of the tetrasialyl-oligosaccharide fraction*

Cirrhotic α_1-AGP was purified from ascitic fluid by an immunoaffinity procedure as described earlier [5]. Normal α_1-AGP was kindly provided by Dr Wickerhauser (American Red Cross, Bethesda). The tetrasialyl-oligosaccharide fractions were obtained by hydrazinolysis of α_1-AGPn and α_1-AGPc followed by anion-exchange HPLC [5,8].

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

Table 1

Carbohydrate composition of α_1-AGPc and α_1-AGPn tetrasialylated oligosaccharides following hydrazinolysis and AX-10 HPLC[a]

	Fuc	Gal	Man	GlcNAc[b]	NeuAc
α_1-AGPc	1.4	4.0	3	4.9	4.3
α_1-AGPn	0.7	4.3	3	5.2	3.9

[a] Calculated on the basis of 3 Man per oligosaccharide
[b] The GlcNAc residue linked to the asparagine residue and transformed to GlcNAcol after hydrazinolysis, re-*N*-acetylation and reduction was not calculated in this table

2.2. *Primary structure analysis*

Permethylation of tetrasialyl-oligosaccharides was performed according to Paz-Parente et al. [9] and the partially methylated monosaccharides were identified by GLC-MS according to Fournet et al. [10].

For ^{1}H-NMR spectroscopic analysis, the oligosaccharide fractions were repeatedly exchanged in D_2O (99.95% atom D, Aldrich) at room temperature with intermediate lyophilisation.

Table 2

Molar ratios of monosaccharide methyl ethers present in the methanolysates of permethylated tetrasialylated oligosaccharide-alditol from α_1-AGPc and α_1-AGPn[a]

Methyl ethers	α_1-AGPc	α_1-AGPn
2,3,4-tri-*O*-Me-Fuc[b]	0.4	0.2
2,3,4,6-tetra-*O*-Me-Gal	0.3	0.1
2,3,4-tri-*O*-Me-Gal	2.3	2.1
2,4,6-tetra-*O*-Me-Gal	1.9	1.9
2,4-di-*O*-Me-Man	0.8	1.1
3,4-di-*O*-Me-Man	1.0	1.0
3,6-di-*O*-Me-Man	1.1	0.9
3,4,6-tri-*O*-Me-Man	0.1	–
3,6-di-*O*-Me-GlcNAcNMe	2.3	3.4
6-*O*-mono Me-GlcNAcNMe	1.1	0.7
1,3,5,6-tetra-*O*-Me-GlcNAcMeol	1.1	0.9
4,7,8,9-tetra-*O*-Me-NeuAc	3.8	3.9

[a] Calculated on the basis of the sum of *O*-Me-Man = 3
[b] Because of the relatively high volatility of this residue, this value was lower than expected

OMe, *O*-methyl; NAcNMe, *N*-acetyl-*N*-methyl; GlcNAcNMeol, *N*-acetyl-*N*-methylglucosaminitol

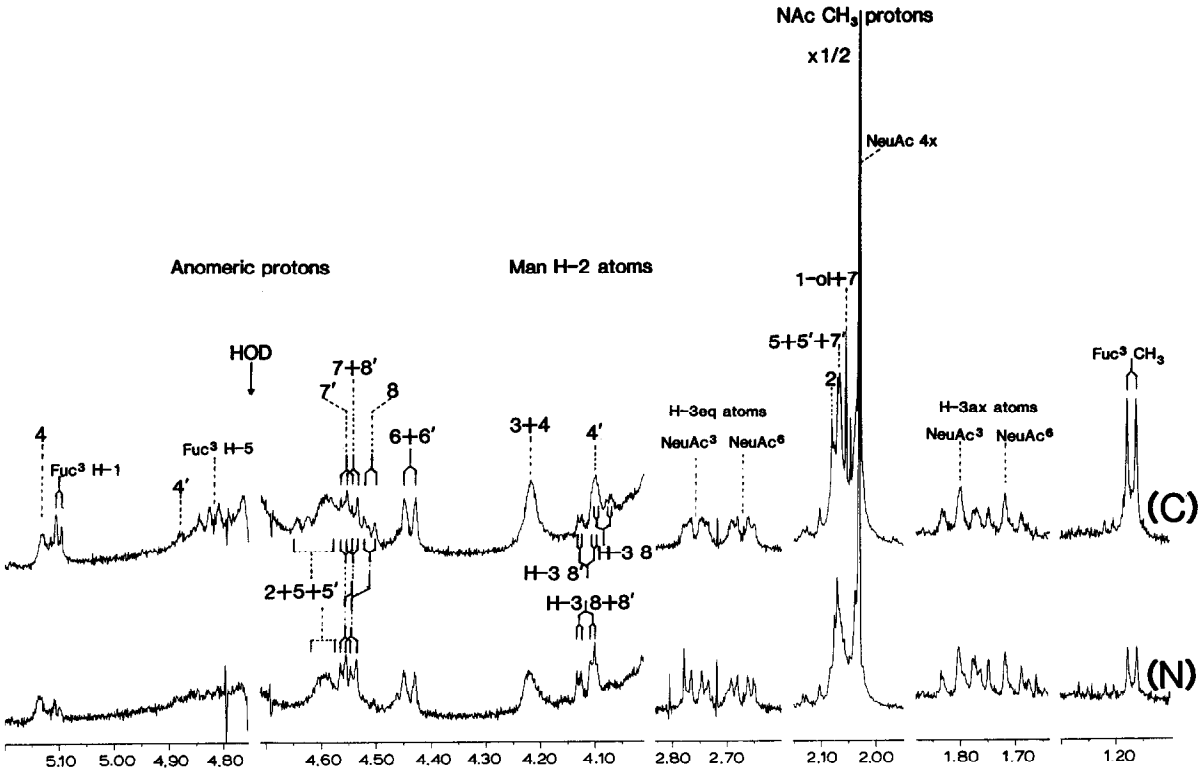
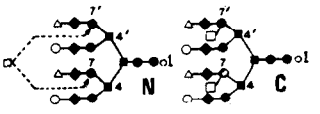


Fig.1. Structural reporter group regions of the resolution-enhanced 400-MHz ^{1}H-NMR spectra of oligosaccharide-alditols from α_1-acid glycoprotein of normal (N) and cirrhotic patient (C), in HO^2H at 300 K.

Table 3

400-MHz ^{1}H chemical shifts of structural reporter groups of constituent monosaccharides for oligosaccharide-alditols from α_1-acid glycoprotein isolated from normal (N) and cirrhotic (C) patients

Reporter group	Residue	Chemical shifts in the following structures[a]	
			
		N	C
H1	GlcNAc-2	4.603	4.636
	Man-3	nd[b]	nd[b]
	Man-4	5.135	5.133
	Man-4′	4.875	4.878
	GlcNAc-5	4.593	4.596
	GlcNAc-5′	4.593	4.596
	Gal-6	4.440	4.440
	Gal-6′	4.440	4.440
	GlcNAc-7	4.555	4.546
	GlcNAc-7′	4.555	4.556
	Gal-8	4.545	4.514
	Gal-8′	4.545	4.546
H-2	Man-3	4.219	4.219
	Man-4	4.219	4.219
	Man-4′	4.103	4.102
H-3	Gal-8	4.115	4.100
	Gal-8′	4.115	4.115
H-3 ax	NeuAc^3	1.801	1.800
	NeuAc^6	1.718	1.718
H-3 eq	NeuAc^3	2.757	2.759
	NeuAc^6	2.672	2.674
NAc	NeuAc^3/NeuAc^6	2.030	2.030
H-1	Fuc^3	5.103	5.103
H-5	Fuc^3	4.822	4.820
CH_3	Fuc^3	1.171	1.170

[a] In the table heading, the structures are represented by a shorthand symbolic notation: ■, Man; ●, GlcNAc; ◆, Gal; □, Fuc; ○, NeuNAc (α2-6); △, NeuNAc (α2-3). For numbering of the monosaccharides and complete structures, see the text

[b] nd, not determined

Spectra were recorded on a Bruker AM-400 WB spectrometer operating in the pulsed Fourier transform mode at a probe temperature of 300 K and equipped with a Bruker Aspect 3000 computer. Chemical shifts (δ) are expressed in ppm downfield from the signal of the methyl of internal acetone (δ = 2.225 ppm in these conditions).

3. RESULTS AND DISCUSSION

The molar carbohydrate composition and methylation analysis of the tetrasialylated oligosaccharides from α_1-AGPc and α_1-AGPn are given in tables 1 and 2, respectively. These results show clearly the tetraantennary structure of these two oligosaccharides with hyperfucosylation, for tetrasialylated fraction from α_1-AGPc (1.4 residue of Fuc in tetrasialylated fraction from α_1-AGPc instead of 0.7 residue of Fuc in tetrasialylated fraction from α_1-AGPn).

In both cases, these fucose residues are located on external GlcNAc residues at the C3 position as demonstrated by the presence of 6-mono-*O*-methyl glucosamine residue and the identification of 1,3,5,6-tetra-*O*-methyl glucosaminitol indicating that the GlcNAc-1 was not fucosylated.

In order to obtain more information on the position of the fucose residue (GlcNAc 5, 5′, 7 or 7′), the two tetrasialylated oligosaccharides were subjected to 400-MHz ^{1}H-NMR spectroscopy.

The NMR spectral data (fig.1) showed for the two oligosaccharide-alditols isolated from α_1-AGPn and α_1-AGPc the presence of a tetraantennary *N*-acetyllactosaminic-type structure. This is demonstrated by the chemical shifts of Man 4 and Man 4′ anomeric protons (δ = 5.133 and 4.878 ppm, respectively) and of H-2 protons of Man 3, 4 and 4′ (δ = 4.219, 4.219 and 4.102 ppm, respectively) (table 3). The intensity of the *N*-acetyl signals (δ = 2.030 ppm) of *N*-acetylneuraminic acid residues indicates that the tetrasialylation of the two oligosaccharide-alditols with α2-3 and α2-6 linkages is in a ratio of 1 to 1. The galactose residues 6 and 6′ are substituted by sialic acid in α2-6 linkage (δH-1 Gal 6: 4.440 and δH-1 Gal 6′: 4.440 ppm) while galactose residues 8 and 8′ are sialylated in α2-3 as demonstrated by chemical shift of Gal 8 anomeric protons (δ = 4.545 for α_1-AGPn, δ = 4.514 for α_1-AGPc), Gal 8′ anomeric protons (δ = 4.545 for α_1-AGPn; δ = 4.546 for α_1-AGPc) and Gal 8 and 8′ H-3 protons: 4.115 for α_1-AGPn and 4.115, 4.100 for α_1-AGPc.

The two oligosaccharide-alditols (from α_1-AGPn and α_1-AGPc) possess the same basic structure see fig.2.

The only difference between NMR spectra (represented at the same scale) of tetrasialylated tetraantennary oligosaccharide-alditols from α_1-AGPn and α_1-AGPc is the presence of one fucose (α_1-AGPn) and two fucose (α_1-AGPc) residues in α1-3 linkage on GlcNAc 7 and 7′. This is demonstrated by chemical shifts of its H-1, H-5

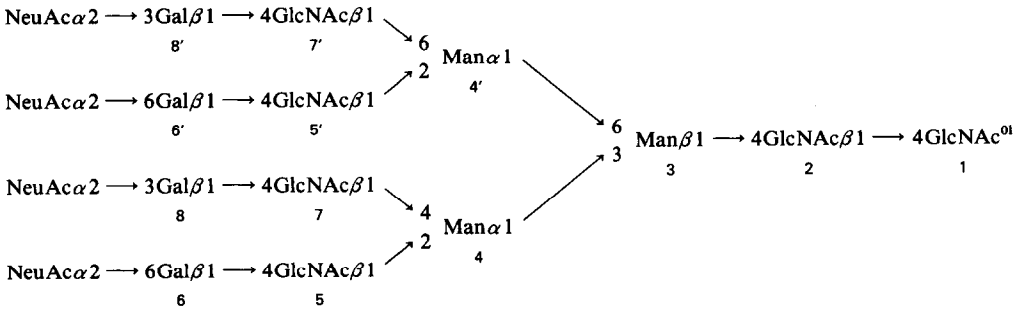


Fig.2.

and CH_3 group (δ = 5.103, 4.820 and 1.170 ppm, respectively). In α_1-AGPn, the single fucose residue is linked to GlcNAc 7 or 7′ while in α_1-AGPc fucose residues are linked to GlcNAc 7 and 7′. This second substitution in the case of tetrasialylated oligosaccharide-alditols from α_1-AGPc is demonstrated by the evaluation of anomeric protons of Gal 8 and GlcNAc 7 (δH-1 = 4.514 and 4.546 ppm, respectively, tentative assignment) and by the upfield shift of *N*-acetyl protons of GlcNAc 7 (δ = 2.055 ppm). Unfortunately we cannot obtain more information about the *N*-acetyl region which appears in a bulk the resolution of which we cannot improve.

On the basis of molar carbohydrate composition, permethylation analysis and 400-MHz ^{1}H-NMR spectroscopy, the structures of fucosylated tetrasialyl-oligosaccharide-alditols isolated from α_1-AGPc and α_1-AGPn are proposed in fig.3. The cirrhotic hyperfucosylation is exclusively located

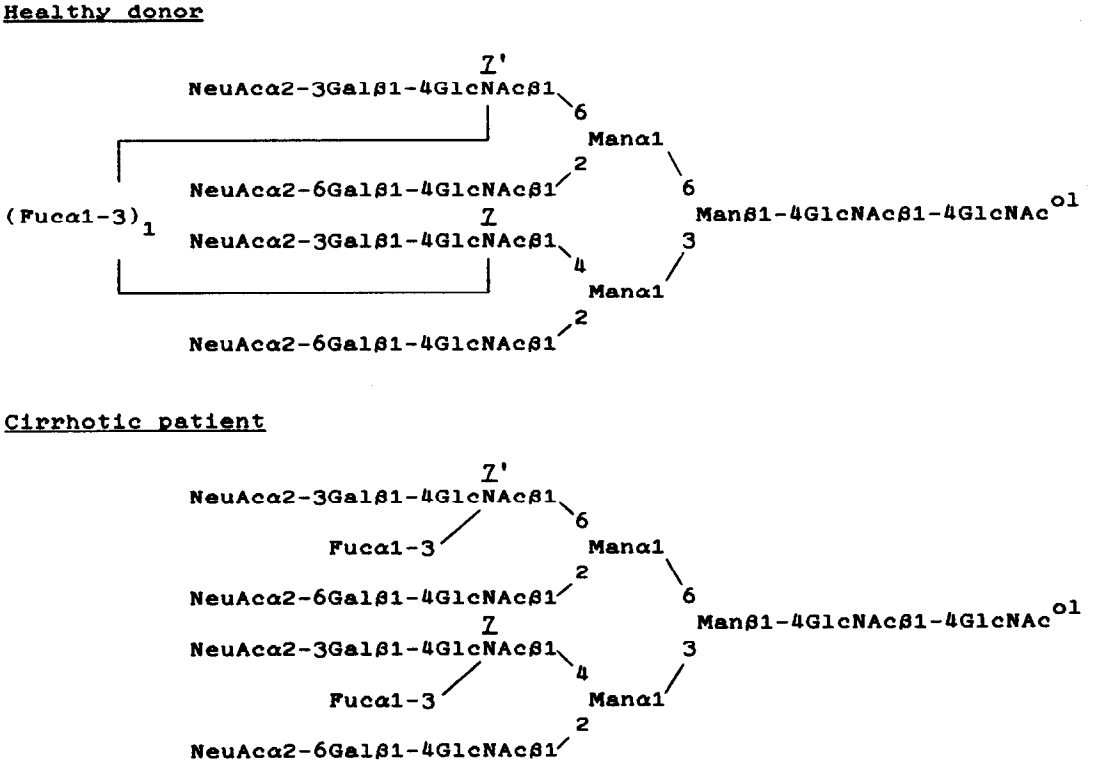


Fig.3. Primary structure of fucosylated tetrasialyl-oligosaccharide-alditols isolated from α_1-AGPn and α_1-AGPc.

external to 7 and 7′ GlcNAc residues corresponding to the sialosyl Lewis X determinant NeuAc (α2-3) Gal (β1-4) [Fuc (α1-3)] GlcNAc. The increase and the precise location of the sialyl Lewis X antigen is described here for the first time in a benign disease such as cirrhosis.

Acknowledgements: This work was supported by grant 857007 from the Institut National de la Santé et de la Recherche Médicale (INSERM).

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