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Structure of Ten Glycopeptides from α_1-Acid Glycoprotein

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The heterogeneity of the carbohydrate moiety of the human plasma α_1-acid glycoprotein has been studied extensively (1-11). However, some ambiguity still exists with regard to the precise structures of the various carbohydrate chains of this glycoprotein. Recently, the careful isolation of 26 glycopeptides in the asialo-form was described (11) and the present study deals with the structure determination of 10 typical representatives of these by means of permethylation analysis, partial acetolysis, and 360-MHz ^{1}H-n.m.r. spectroscopy.

MATERIALS AND METHODS

α_1-Acid glycoprotein was isolated from a pool of normal human plasma (12). Asialoglycopeptides were fractionated as described in a previous paper (11). The molar ratios of hexoses and *N*-acetylhexosamines were determined by g.l.c. (13). Methylation was performed according to Hakomori (14), and the partial methylated monosaccharides were identified as their acetylated methyl glycoside derivatives (15,16). Acetolysis (17) was carried out with a reaction time of 3 h. After reduction and permethylation, the oligosaccharides were identified by g.l.c.-m.s. (mass fragmentography at *m/e* 236, 260, and 277, Riber model apparatus) (18). ^{1}H-N.m.r. spectroscopy was performed at 360 MHz on a Bruker HX-360 spectrometer operating in the Fourier-transform mode at probe temperatures of 25°C and 60°C. Chemical shifts are given relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (indirectly to acetone in D_2O: 2.225 p.p.m.).

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Table I. Carbohydrate Composition of 10 Pronase-glycopeptides Derived from Desialylated Reduced, and Carboxymethylated α_1-acid Glycoprotein

Glycopeptide	Carbohydrate compound[a]						
	Fuc	Gal	Man	Gal/Man[b]	GlcN	Hex/HexN[b]	Total
Class A							
GP II-6	0	1.9(2)	3.0(3)	0.7	3.97(4)	1.25	9
Class B							
GP II-5	0	2.7(3)	3.02(3)	1	5.1(5)	1.2	11
GP III-7	0	3.0(3)	3.0(3)	1	4.94(5)	1.2	11
GP V-5	0	3.0(3)	3.0(3)	1	4.99(5)	1.2	11
Class C							
GP III-6	0	3.82(4)	3.0(3)	1.3	5.99(6)	1.17	13
GP V-4	0	3.54(4)	3.0(3)	1.3	5.73(6)	1.17	13
Class D							
GP III-5	0.85(1)	4.09(4)	3.0(3)	1.3	5.64(6)	1.17	14
GP V-2	0.91(1)	3.5(4)	3.0(3)	1.3	6.2(6)	1.17	14
GP V-3	1.12(1)	4.06(4)	3.0(3)	1.3	5.8(6)	1.17	14
GP V-1	0.70(1)	3.86(4)	3.0(3)	1.3	4.76(5)	1.4	13

[a] *Expressed in mol of monosaccharides per mol of glycopeptides*

[b] *Molar ratio of hexose to hexosamine*

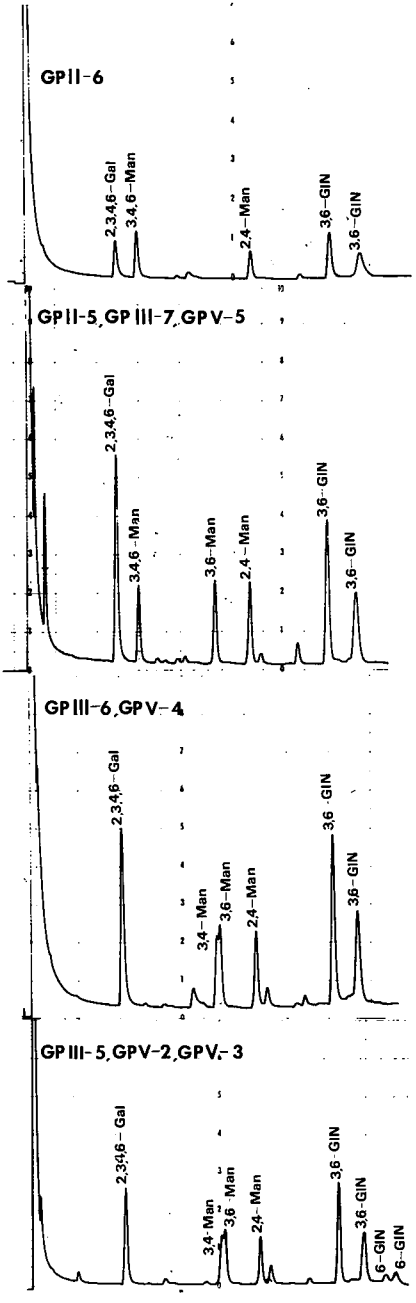

Fig. 1. Gas chromatography of acetylated monosaccharide methyl ethers obtained by methanolysis of permethylated glycopeptides and peracetylation. Aerograph 1200 apparatus, glass column (0.2 x 300 cm), 3% Carbowax 6000 on Chromosorb W-HMDS (60-80 mesh), 110°-200°C (2°C/min), nitrogen: 30 ml/min.

Table II. Identification and Molar Ratios of Monosaccharide Methyl Ethers Present in the Methanolyzates of Ten Permethylated Asialoglycopeptides from α_1-Acid Glycoprotein

	Methylated monosaccharides			
Glycopeptide	2,3,4-tri-O-methyl-fucose	2,3,4,6-tetra-O-methyl-galactose	3,4,6-tri-O-methyl-mannose	3,4-di-O-methyl-mannose
Class A				
GP II-6	0	1.88(2)	1.88(2)	0
Class B				
GP II-5	0	3.06(3)	1.02(1)	0
GP III-7	0	2.85(3)	1.07(1)	0
GP V-5	0	3.12(3)	1.2(1)	0
Class C				
GP III-6	0	3.76(4)	0	0.95(1)
GP V-4	0	3.85(4)	0	0.97(1)
Class D				
GP III-5	0.84(1)	3.83(4)	0	0.88(1)
GP V-2	0.68(1)	3.81(4)	0	1.01(1)
GP V-3	0.82(1)	3.75(4)	0	1.04(1)
GP V-1	0.51(1)	4.3(4)	0	0.87(1)

[a] *Expressed in mol of monosaccharides per mole of glycopeptides*

2,4-di-O-methyl-mannose	*3,6-di-O-methyl-mannose*	*3,6-di-O-methyl-glucosamine*	*6-mono-O-methyl-glucosamine*	*Total*
1.0(1)	*0*	*4.2(4)*	*0*	*9*
0.97(1)	*0.89(1)*	*4.55(1)*	*0*	*11*
1.05(1)	*1.09(1)*	*4.5(5)*	*0*	*11*
1.0(1)	*0.88(1)*	*4.99(5)*	*0*	*11*
1.0(1)	*0.99(1)*	*5.54(6)*	*0*	*13*
1.0(1)	*0.81(1)*	*5.5(6)*	*0*	*13*
1.0(1)	*0.96(1)*	*4.94(5)*	*0.80(1)*	*14*
1.0(1)	*0.91(1)*	*5.2(5)*	*0.6(1)*	*14*
0.88(1)	*0.77(1)*	*4.86(5)*	*0.52(1)*	*14*
1.0(1)	*0.84(1)*	*3.87(4)*	*0.43(1)*	*13*

RESULTS AND DISCUSSION

The molar carbohydrate compositions of the 10 compounds are reported in Table I. According to the number of the various monosaccharide constituents, the glycopeptides can be divided into 4 classes A, B, C, and D (A=GP II-6; B=GP II-5, GP III-7, and GP V-5; C=GP III-6 and GP V-4; D=GP III-5, GP V-2, and GP V-3). GP V-1 is a reducing oligosaccharide. The data of methylation analysis are represented in Table II and Fig. 1. The same methylated neutral sugars in identical molar ratios were found in all glycopeptides belonging to one class. The oligosaccharides isolated from the acetolyzate of GP V-5 are: β-Gal-(1→4)-GlcNAc, β-GlcNAc-(1→2)-Man and β-GlcNAc-(1→4)-α-Man-(1→3)-Man. The presence of the trisaccharide demonstrates that the third residue of *N*-acetyllactosamine is linked to the mannose residue 4.

The glycopeptide structures of the classes A-D would be characterized by 360-MHz ^{1}H-n.m.r. spectroscopy. Some relevant data are given in Table III.

Table III. Chemical Shifts of H-1 and H-2 Mannose Residues of the Bi-, Tri-, and Tetraantennary Structures

Structure	*H-1 of D-mannose*			*H-2 of D-mannose*		
	3	*4*	*4'*	*3*	*4*	*4'*
Biantennary	*4.77*	*5.12*	*4.93*	*4.24*	*4.18*	*4.11*
Triantennary	*4.76*	*5.12*	*4.93*	*4.21*	*4.21*	*4.11*
Tetraantennary	*4.77*	*5.12*	*4.86*	*4.22*	*4.22*	*4.09*

Class A glycopeptide has a biantennary structure that is identical to the asialoglycan of human serotransferrin (19,20). The characteristic n.m.r. features of the mannotrioside branching-core substituted by GlcNAc residues 2, 5, and 5' are the chemical shifts of the mannose residue H-1 and H-2, as given in Table III. Glycopeptides of classes B and C have tri- and tetraantennary structures, respectively, which means the attachment of one or two additional *N*-acetyllactosamine residues to the mannotrioside core of the biantennary structure. The occurrence of these extra branches is expressed in the shift increments of the mannose residue H-1 and H-2 (Table III).

Class D glycopeptides consist of a tetraantennary glycan chain with an extra fucose residue. The type of linkage of this fucose residue to a GlcNAc residue 7 follows from the chemical shift of the fucose residue H-5 (4.84 p.p.m.) and from the shift increment of the *N*-acetyl signal of GlcNAc residue 7 (2.080→2.068 p.p.m.).

The combination of the results of methylation analysis, partial acetolysis, and 360-MHz ^{1}H-n.m.r. spectroscopy leads to the conclusions that in the triantennary structures the additional *N*-acetyllactosamine is coupled *via* a β-(1→4) linkage to a mannose residue 4 whereas in the tetraantennary structures the additional *N*-acetyllactosamine residues are coupled *via* β-(1→4) and β-(1→6) linkages to mannose residues 4 and 4', respectively (see J. Montreuil and J. F. G. Vliegenthart in this volume).

REFERENCES

1. Jeanloz, R. W., *in* "Glycoproteins" (A. Gottschalk, ed.), p. 565, American Elsevier Publish. Co., New York (1972).
2. Spiro, R. G., *Adv. Prot. Chem. 27*, 350 (1973).
3. Kornfeld, R., and Kornfeld, S., *Annu. Rev. Biochem. 45*, 217 (1976).
4. Montreuil, J., *Pure Appl. Chem. 42*, 431 (1975).
5. Wagh, P. V., Bornstein, I., and Winzler, R. J., *J. Biol. Chem. 244*, 658 (1969).
6. Hatcher, V. B., and Jeanloz, R. W., *Colloq. Int. C.N.R.S. 221*, 329 (1973).
7. Schwarzmann, G., Reinhold, V., and Jeanloz, R. W., *Colloq. Int. C.N.R.S. 221*, 85 (1973).
8. Fournet, B., Takerkart, G., Brohon, J., and Montreuil, J., *Bull. Soc. Chim. Biol. 50*, 1352 (1968).
9. Bayard, B., and Montreuil, J., *Arch. Int. Physiol. Biochim. 77*, 564 (1969).
10. Bayard, B., and Fournet, B., *Carbohydr. Res. 46*, 75 (1976).
11. Schmid, K., Nimberg, R. B., Kimura, A., Yamaguchi, H., and Binette, J. P., *Biochim. Biophys. Acta 492*, 291 (1977).
12. Schmid, K., *in* "The Plasma Proteins" (F. W. Putman, ed.), vol. 1, p. 184, Academic Press, New York (1975).
13. Zanetta, J. P., Brechenridge, W. C., and Vincendon, G., *J. Chromatogr. 69*, 291 (1972).
14. Hakomori, S. I., *J. Biochem. (Tokyo) 55*, 205 (1964).
15. Fournet, B., Leroy, Y., Montreuil, J., and Mayer, H., *Colloq. Int. C.N.R.S. 221*, 111 (1973).
16. Fournet, B., Thesis, Lille (1973).
17. Bayard, B., and Montreuil, J., *Carbohydr. Res. 24*, 427 (1972).

18. Fournet, B., Dhalluin, J. M., Strecker, G., and Montreuil, J., in preparation.
19. Spik, G., Bayard, B., Fournet, B., Strecker, G., Bouquelet, S., and Montreuil, J., *FEBS Lett. 50*, 296 (1975).
20. Dorland, L., Haverkamp, J, Schut, B. L., Vliegenthart, J. F. G., Spik, G., Strecker, G., Fournet, B., and Montreuil, J., *FEBS Lett. 77*, 15 (1977).