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THE PRIMARY STRUCTURE OF THE ASIALO-CARBOHYDRATE UNITS OF THE FIRST GLYCOSYLATION SITE OF HUMAN PLASMA α_1-ACID GLYCOPROTEIN

K. SCHMID [a], J.P. BINETTE [a], L. DORLAND [b], J.F.G. VLIEGENTHART [b], B. FOURNET [c] and J. MONTREUIL [c]

[a] *Department of Biochemistry, Boston University School of Medicine, Boston University Medical Center, Boston, MA (U.S.A.),* [b] *Department of Bio-organic Chemistry, University of Utrecht, Utrecht (The Netherlands), and* [c] *Laboratoire de Chimie Biologique, Université des Sciences et Techniques de Lille I, Villeneuve d'Ascq (France)*

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Summary

The elucidation of the structures of the carbohydrate units linked to glycosylation site I of human plasma α_1-acid glycoprotein is described. These carbohydrate units can be grouped into compounds with bi- (class A) and tri-antennary (class B) structures and the triantennary structure with a fucose residue (class BF) (Fig. 1). The structural variability of the carbohydrate units of glycosylation site I and also of glycosylation sites II to V (Fournet, B., Montreuil, J., Strecker, G., Dorland, L., Haverkamp, J., Vliegenthart, J.F.G., Binette, J.P. and Schmid, K. (1978) Biochemistry 17, 5206—5214) accounts largely for the microheterogeneity of α_1-acid glycoprotein.

The carbohydrate moiety of human plasma α_1-acid glycoprotein [1] represents a classical example of the microheterogeneity which frequently occurs in glycoproteins. To define the structure of the carbohydrate chains of this protein, Schmid et al. [2] prepared a series of asialoglycopeptides derived from each of its five glycosylation sites. Recently, Fournet et al. [3] elucidated the primary carbohydrate structure of the glycopeptides isolated from the glycosylation sites II, III, IV and V* of this plasma globulin. These carbohydrate units were found to be N-glycosidically linked and possessed bi-, tri-

*For the designation of the glycosylation sites and the glycopeptides of α_1-acid glycoprotein, the same nomenclature was used as that described previously [2,3].

TABLE I

THE CARBOHYDRATE COMPOSITION OF GLYCOPEPTIDES DERIVED FROM ASIALO-α_1-ACID GLYCOPROTEIN

The number of Man residues per carbohydrate chain was assumed to be 3.0.

Glycopeptide		Monosaccharide residues (mol/mol of glycopeptide)				
		Fuc	Gal	Man	GlcNAc	Total number of residues
Class A	GPI-8	0	2.1(2)	3.0	3.7(4)	9
Class B	GPI-3	0	3.1(3)	3.0	4.8(5)	11
	GPI-6	0	3.1(3)	3.0	4.8(5)	11
	GPI-7	0	2.8(3)	3.0	4.9(5)	11
Class BF	GPI-2	0.6(1)	2.6(3)	3.0	5.1(5)	12
	GPI-5	0.7(1)	2.9(3)	3.0	4.5(5)	12

and tetraantennary structures. In the latter two classes of compounds a Fuc residue may be present and is then linked in an $\alpha(1\rightarrow3)$ bond to GlcNAc residue 7 (Fig. 1).

In this paper we report on the structures of the carbohydrate units from glycosylation site I of α_1-acid glycoprotein. For this study methylation analysis and 360 MHz ^{1}H-NMR spectroscopy were employed [3]. The asialo-glycopeptides from the latter glycosylation site were obtained as described earlier [2], and the carbohydrate compositions of these glycopeptides were determined by gas-liquid chromatography [3] (Table I). On the basis of their chemical compositions, these compounds were grouped into classes A, B and BF.

The results of the methylation analysis are presented in Table II. The chemical shifts of the anomeric and certain structurally relevant non-anomeric protons obtained from the 360 MHz ^{1}H-NMR spectra are listed in Table III.

The class A glycopeptide was found to have a biantennary structure (Fig. 1). The analytical data for this compound agree with those reported earlier for the corresponding carbohydrate unit derived from glycosylation site II of α_1-acid glycoprotein [3] and from human serotransferrin [4]. While generally 2 mg of a homogeneous glycopeptide is preferred for NMR analysis, it should be noted that, although only 0.05 mg of GP-I-8 was available, the analysis was carried out successfully.

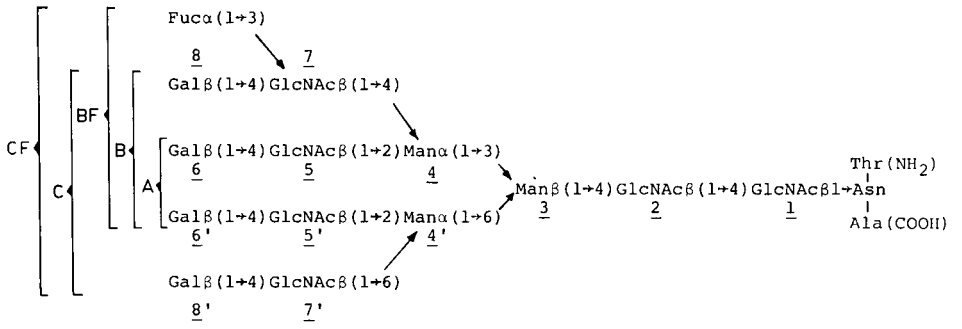

Fig. 1. The primary structures of classes A, B and BF carbohydrate units of glycosylation site I of human plasma α_1-acid glycoprotein. The tetraantennary structures occurring in the other glycosylation sites of this protein are designated with C and CF.

TABLE II

MOLAR RATIOS OF MONOSACCHARIDE METHYL ETHERS PRESENT IN THE METHANOLYSATES OF THE PERMETHYLATED GLYCOPEPTIDES DERIVED FROM ASIALO-α_1-ACID GLYCOPROTEIN

Glycopeptide		Methylated monosaccharides (mol/mol of glycopeptide)							Total number of residues	Amino acids
		2,3,4-tri-OMe-Fuc	2,3,4,6-tetra-OMe-Gal	3,4,6-tri-OMe-Man	2,4-di-OMe-Man	3,6--diOMe-Man	3,6-diO-Me-GlcNAc-NMe	6-OMe-GlcNAc-NMe		
Class A	GPI-8	0	2.1(2)	1.6(2)	1.0(1)	0	3.5(4)	0	9	Asn
Class B	GPI-3	0	2.8(3)	1.4(1)	1.0(1)	0.9(1)	4.7(5)	0	11	Asn
	GPI-6	0	2.6(3)	0.8(1)	1.0(1)	1.0(1)	4.6(5)	0	11	Thr-Asn
	GPI-7	0	2.8(3)	1.1(1)	1.0(1)	1.0(1)	4.3(5)	0	11	Thr-Asn-Ala
Class BF	GPI-2	0.26(1)	3.0(3)	1.0(1)	1.0(1)	1.0(1)	3.6(4)	0.7(1)	12	Asn
	GPI-5	0.5(1)	2.8(3)	0.8(1)	1.1(1)	1.0(1)	3.9(4)	0.6(1)	12	Thr-Asn

TABLE III

CHEMICAL SHIFTS OF CHARACTERISTIC PROTONS OF CONSTITUENT MONOSACCHARIDES OF GLYCOSYLATION SITE I GLYCOPEPTIDES DERIVED FROM α_1-ACID GLYCOPROTEIN

Compound		Chemical shift of H-1 of residue										
		GlcNAc 1	GlcNAc 2	Man 3	Man 4	Man 4′	GlcNAc 5	GlcNAc 5′	Gal 6	Gal 6′	GlcNAc 7	Gal 8
Class A	GPI-8	5.041	4.616	~4.77	5.117	4.926	4.581	4.581	4.471	4.471	—	—
Class B	GPI-3	5.070	4.616	4.756	5.114	4.924	4.583	4.561	4.468	4.468	4.539	4.468
	GPI-6	5.044	4.611	4.754	5.113	4.922	4.582	4.561	4.468	4.468	4.541	4.468
	GPI-7	5.089	4.610	4.755	5.116	4.923	4.584	4.560	4.469	4.469	4.542	4.469
Class BF	GPI-2	~5.07	4.612	4.760	5.115	4.925	~4.58	~4.58	~4.47	~4.47	~4.54	~4.47
	GPI-5	5.044	4.611	4.755	5.111	4.922	4.583	4.583	4.471	4.471	4.548	4.441

		Chemical shift of H-2 of residue			Chemical shift of the N-acetyl CH_3 of residue					Chemical shift of fucose protons		
		Man 3	Man 4	Man 4′	GlcNAc 1	GlcNAc 2	GlcNAc 5	GlcNAc 5′	GlcNAc 7	H-1	H-5	H-6
Class A	GPI-8	4.253	4.192	4.113	2.013	2.079	2.049	2.047	—	—	—	—
Class B	GPI-3	4.215	4.215	4.110	2.013	2.077	2.047	2.047	2.077	—	—	—
	GPI-6	4.214	4.214	4.107	2.008	2.076	2.045	2.045	2.077	—	—	—
	GPI-7	4.215	4.215	4.109	2.005	2.077	2.049	2.046	2.077	—	—	—
Class BF	GPI-2	4.215	4.215	4.109	2.015	2.078	2.048	2.048	2.068	5.115		1.180
	GPI-5	4.214	4.214	4.110	2.008	2.078	2.049	2.049	2.071	5.111	4.844	1.176

The class B glycopeptides comprised three compounds which differed only in their amino acid compositions. Their carbohydrate chains were identified as a triantennary structure (Fig. 1). Methylation analysis demonstrated that, in comparison to the class A structure, one of the Man residues is substituted both in position 2 and 4 (Table II) and that an additional Gal and GlcNAc residue are present. The former residue is terminally located and the latter is substituted on carbon atom 4 of Man. Also in contrast to the biantennary structure (class A) the ^{1}H-NMR data revealed an additional N-acetyl lactosamine unit linked to Man residue 4. Further, the chemical shifts of H-1 and H-2 of the mannose residues are typical for the substitution pattern of the trimannoside core [5].

The class BF glycopeptides consist of two compounds which can be distinguished from each other by their amino acid compositions. Their carbohydrate moieties differ from that of class B only by an additional Fuc residue. Judging from the methylation results this residue occupies a terminal position and is linked to GlcNAc in position 3. The ^{1}H-NMR data demonstrated that the carbohydrate units possess a triantennary structure extended with a Fuc linked in an $\alpha(1\rightarrow3)$ bond to GlcNAc 7.

When comparing the data described in the present study with those of our earlier investigation on the other glycosylation sites [3], it is noteworthy that the tetraantennary structure with or without fucose, which has been shown to occur in glycosylation sites II, III, IV and V, is absent in the first glycosylation site of α_1-acid glycoprotein. These observations illustrate that each glycosylation site has its individual type of microheterogeneity.

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