STUDIES ON GLYCOCONJUGATES. LXIV. COMPLETE STRUCTURE OF TWO CARBOHYDRATE UNITS OF HUMAN SEROTRANSFERRIN

G. SPIK, B. BAYARD, B. FOURNET, G. STRECKER, S. BOUQUELET and J. MONTREUIL

Laboratoire de Chimie Biologique, Université des Sciences et Techniques de Lille I et Laboratoire Associé au C.N.R.S. n° 217, B.P. n° 36, Villeneuve d'Ascq, France

and

Institut de Recherches sur le Cancer de Lille (Institut Jules Driessens) et U 124 de l'I.N.S.E.R.M., B.P. n° 3567, 59020 – Lille Cédex, France

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1. Introduction

In previous papers [1–4] we have described 2 glycopeptides (Carbohydrate → Asn–Lys and Ser–Asn ← Carbohydrate) isolated from pronase digests of human asialo-serotransferrin and we have demonstrated that in both glycopeptides the glycans were bound through a 4-*N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine linkage. Evidence that the 2 glycans proceed from two different parts of the polypeptide chain was demonstrated by determination of amino-acid sequences of tryptic and chymotryptic glycopeptides : glycan I is conjugated in the N-terminal part of the peptidic chain and corresponds to the dipeptide Asn–Lys whereas glycan II is conjugated in the C-terminal part and corresponds to the dipeptide Ser–Asn [5–8]. In this paper we report the primary structure of glycans I and II determined by application of different methods: exhaustive methylation, mild hydrolysis, acetolysis, hydrazinolysis as well as use of specific glycosidases.

2. Materials and methods

Human serotransferrin was isolated according to Roop and Putnam [9]. The glycopeptides isolated from pronase digestion [4] were submitted to a free flow continuous electrophoresis (Elphor–Vap II apparatus) carried out at pH 2.4 in 0.5 N acetic acid at 1700 V. Homogeneity of the glycopeptides was verified by paper electrophoresis at pH 2.4 (1 N acetic acid).

Monosaccharides were determined by classical colorimetric analysis [10] and by gas-liquid chromatography [11].

Exhaustive methylation was performed according to Hakomori [12] on native and desialyzed glycopeptides and the methyl ethers of neutral monosaccharides and of *N*-methyl glucosamine were identified according to Fournet et al. [13,14]. Mild acid hydrolysis was carried out with Dowex 50 x 8 (25–50 mesh; H^+) at 100°C during 1 hr [15]. Acetolysis and separation of different oligosaccharides were performed according to Bayard et al. [16,17]. Selective cleavage of acetyl-hexosamine linkages by hydrazinolysis-nitrous deamination and determination of the relative proportions of liberated derivatives reduced by sodium tritated borohydride and separated by paper chromatography were carried out according to Bayard and Montreuil [18]. Enzymatic digestions were realized with neuraminidase (EC 3.2.1.18) from *Clostridium perfringens* [19], β-galactosidase (EC 3.2.1.23) and *N*-acetyl-β-hexosaminidase (EC 3.2.1.30) from ox spleen [20], α-mannosidase (EC 3.2.1.24) from jack bean meal [21] and β-mannosidase (EC 3.2.1.-) from crude pineapple bromelain [22]. Amino acid sequences were determined as previously described [4].

3. Results

3.1. *Isolation and identification of glycopeptides*

Free flow electrophoresis of pronase digests of

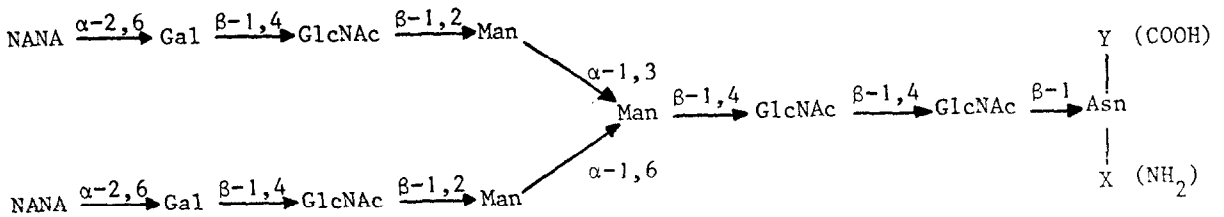

Fig.1. Structure of the carbohydrate unit of the glycopeptides GP-2 and GP-3 isolated from human serotransferrin. In glycopepfide GP-2, X = Ser, Y = none; in glycopeptide GP-3, X = none, Y = Lys.

native serotransferrin leads to the isolation of four glycopeptidic fractions termed GP-1 to GP-4. Table 1 shows, i) that the amino acid sequences of GP-1 and GP-2 are identical and related to the Ser–Asn ← Carbohydrate glycopeptide, ii) that the amino acid sequences of GP-3 and GP-4 are also identical and correspond to the Carbohydrate → Ans–Lys glycodipeptide.

3.2. *Carbohydrate composition*

Percent and molar carbohydrate compositions of the four glycopeptides are reported in table 1. Carbohydrate compositions of GP-2 and GP-3 are identical, GP-4 contains 1 *N*-acetylneuraminic acid residue less, GP-1 contains 1 galactose, 1 *N*-acetylglucosamine and 1 *N*-acetylneuraminic acid residues more. Structural studies we report below concern only the glycans of GP-2 and GP-3 sialo- and asialo-glycopeptides.

Table 1
Centesimal and molar carbohydrate and amino acid compositions of the four glycopeptides isolated by free flow continuous electrophoresis from pronase hydrolysates of human serotransferrin

Compounds	GP-1	GP-2	GP-3	GP-4
Galactose	3	2.4	2.3	2
Mannose	3	3	3	3
N-acetylglucosamine	5	4	4	4
N-acetylneuraminic acid	3	2	2	1
Aspartic acid	0.90	0.80	1.05	0.90
Serine	0.80	0.87	0	0
Lysine	0	0	0.80	0.80
N-terminal	Ser	Ser	Asn	Asn
C-terminal	Asn	Asn	Lys	Lys

Table 2
Identification and determination* of methylated monosaccharides obtained from permethylated glycopeptides GP-2 and GP-3

Glycopeptides	Methylated monosaccharides			
	2,3,4,6-Gal	3,4,6-Man	2,4-Man	2,3,4-Gal
Sialo-GP-2	0	1.7	1	2.04
Asialo-GP-2	1.85	1.85	1	0
Sialo-GP-3	0	2.24	1	2.09
Asialo-GP-3	1.90	1.62	1	0

* On the basis of one 2,4-di-*O*-methylmannose residue.

3.3. *Methylation*

Exhaustive methylation (table 2) of sialo- and asialo-glycopeptides GP-2 and GP-3 shows that i) the same methylated neutral sugars in identical molar ratios are found in both glycopeptides; ii) the galactose residues are substituted in 6-position by sialic acid residues; iii) only 3,6-di-O-methyl-*N*-methylglucosamine has been identified.

3.4. *Mild acid hydrolysis*

Among the numerous oligosaccharides obtained by Dowex 50 X 8 hydrolysis, the following compounds have been isolated and identified: Galβ1 → 4 GlcNAc, Manα 1 → 3 Man, Manα 1 → 6 Man and Manα 1 → 3 [Manα 1 → 6] Man.

3.5. *Acetolysis*

The more interesting oligosaccharides isolated from acetolysates of sialo-GP-2 and -GP-3 are the following: Galβ1 → 4 GlcNAc, GlcNAcβ1 → 2 Man, Manβ1 → 4GlcNAc, NANAα2 → 6 Galβ1 → 4 GlcNAcβ1 → 2 Man, NANAα2 → 6 Galβ1 → 4 GlcNAcβ1 → 2 Manα1 → 3 Man.

3.6. *Hydrazinolysis-nitrous deamination*

Hydrazinolysis followed by nitrous deamination of asialo-GP-2 and GP-3 leads to the formation of 3 components which were isolated by preparative paper chromatography and identified as 2,5-anhydro-mannose (anhMan), Galβ1 → 4 anhMan and Manα1 → 3 [Manα1 → 6] Manβ1 → 4 anhMan in a 1:2:1 molar ratio. As no *N*-acetylglucosamine residue was found in external position by methylation and by enzymatic hydrolysis, the presence of free 2,5-anhydro-mannose demonstrates that the sequence GlcNAc → GlncNAc is present in both glycans. The β-mannosidic linkage has been proved by use of a β-mannosidase devoid of α-mannosidase activity.

4. Conclusion

On the basis of these results we can propose the following structure for the carbohydrate moieties of GP-2 and GP-3 glycopeptides. This structure is quite different from that proposed by Jamieson et al. [23] which was established on the basis of the presence of 4 mannose residues. It shows many similarities with the structure of the glycans isolated from IgE by Baenziger and Kornfeld [24] and from lactotransferrin [25].

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