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STRUCTURE DETERMINATION OF TWO OLIGOMANNOSIDE-TYPE GLYCOPEPTIDES OBTAINED FROM BOVINE LACTOTRANSFERRIN, BY 500 MHz ^{1}H-NMR SPECTROSCOPY

H. VAN HALBEEK [a,*], L. DORLAND [a], J.F.G. VLIEGENTHART [a], G. SPIK [b], A. CHERON [b] and J. MONTREUIL [b]

[a] *Department of Bio-Organic Chemistry, University of Utrecht, Croesestraat 79, 3522 AD Utrecht (The Netherlands) and* [b] *Laboratoire de Chimie Biologique, Université des Sciences et Techniques de Lille I, Villeneuve d'Ascq (France)*

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The elucidation of the structures of two carbohydrate units, *N*-glycosidically linked to an asparagine residue of bovine lactotransferrin, is described. These carbohydrate structures are of the oligomannoside type and contain eight or nine mannose residues, respectively. The potency of 500 MHz ^{1}H-NMR spectroscopy in primary structure determination of two closely related carbohydrate chains present in a mixture is demonstrated. This implies that 500 MHz ^{1}H-NMR spectroscopy can disclose microheterogeneity which is almost untraceable using other approaches.

As we have shown recently, 500 MHz ^{1}H-NMR spectroscopy is a powerful method in the structure analysis of carbohydrate chains of various glycoproteins [1–4]. For example, the spectra of oligomannoside-type glycoasparagines and oligosaccharides could be interpreted in terms of structural assignments up to and including an oligosaccharide containing nine mannose residues [3,4].

In this paper we report on the structures of the carbohydrate unit of bovine lactotransferrin. Glycopeptides from this glycoprotein were obtained as described elsewhere [5] and the carbohydrate composition of the resulting preparations was determined by gas-liquid chromatography [6]. On the basis of the chemical compositions (e.g., 2.0 mol GlcNAc and 8.6 mol mannose per mol of the largest glycopeptide obtained), the glycopeptide mixtures were grouped into the class of oligomannoside-type asparagine-bound structures. Methylation analysis of the largest glycopeptide fraction afforded a mixture of the methylglycosides of 2,4-di-*O*-methyl-mannose, 3,4,6-tri-*O*-methyl-mannose, 2,3,4,6,-tetra-*O*-methyl-mannose and 2,6-di-*O*-methyl-*N*-methyl-GlcNAc in a ratio 2.0 : 2.9 : 3.2 : 1.7 [5] giving rise to a tentative structure for the carbohydrate moiety of the glycopeptide containing nine mannose residues (see also Ref. 7).

To elucidate unambiguously the primary structure, the largest glycopeptide preparation was subjected to 500 MHz ^{1}H-NMR spectroscopy in ^2H_2O at several probe temperatures (for experimental details see Ref. 1). The 500 MHz ^{1}H-NMR spectrum is depicted in Fig. 1. In Table I the chemical shifts of the anomeric and certain structurally relevant non-anomeric protons are listed, together with those of the oligosaccharide containing nine mannose residues, isolated from urine of mannosidosis patients [4].

The above mentioned glycopeptide mixture from bovine lactotransferrin was found to consist mainly of two components, as is evident from the spectral interpretation outlined below. The oligomannan part of the largest one of these two has the same structure as reported for the reference mannosidosis

* To whom correspondence should be addressed.

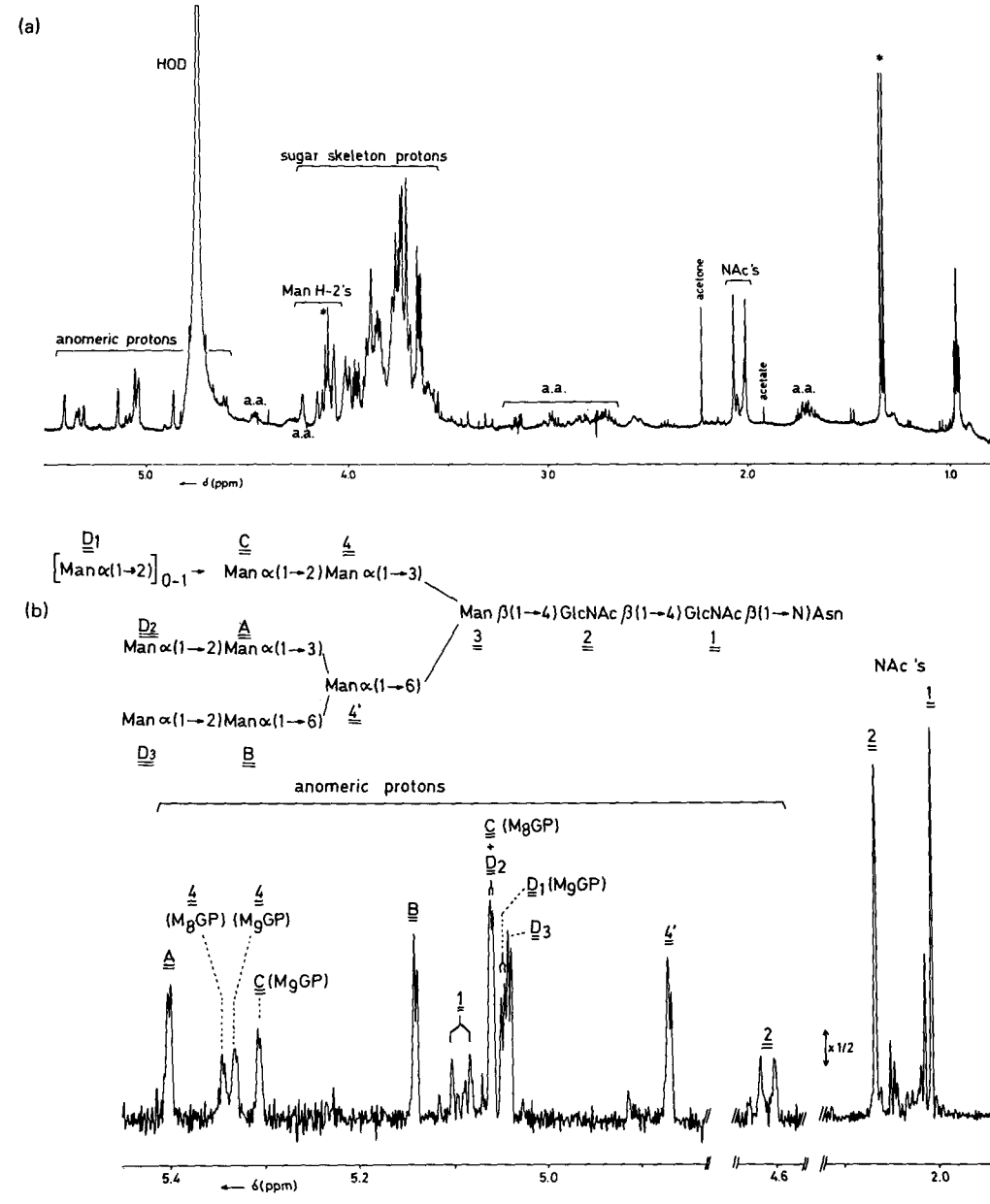

Fig. 1. 500 MHz ^{1}H-NMR spectrum of the mixture of glycopeptides M_8GP and M_9GP (ratio 2 : 3) from bovine lactotransferrin, in ^2H_2O at 300 K, together with their structures. (a) Overall spectrum; a.a. denotes signal of amino-acid proton. The signals indicated by an asterisk stem from a non-protein, non-carbohydrate contaminant. (b) Expanded, resolution-enhanced anomeric region and *N*-acetyl region of the spectrum; the numbers and letters in the spectrum refer to the corresponding residues in the structure. The relative intensity of the *N*-acetyl region deviates from that of the other part of the spectrum, as indicated.

oligosaccharide (M_9G). The NMR data for the mannose anomeric protons of the glycopeptide, designated M_9GP, agree with those of the β-anomer of M_9G, as can be seen from Table I.

However, the H-1 doublet of mannose 4 at δ = 5.333 ppm for M_9GP is accompanied by an identi-

TABLE I

^1H CHEMICAL SHIFTS OF PERTINENT STRUCTURAL REPORTER GROUPS OF CONSTITUENT MONOSACCHARIDES FOR TWO OLIGOMANNOSIDE-TYPE GLYCOPEPTIDES (M_8GP AND M_9GP) FROM BOVINE LACTOTRANSFERRIN AND FOR A URINARY MANNOSIDOSIS OLIGOSACCHARIDE (M_9G) AS REFERENCE COMPOUND [4]

Chemical shifts are given at 300 K, relative to sodium 2,2-dimethyl-2-silapentane-5-sulphonate in ^2H_2O (but were actually measured relative to internal acetone: $\delta = 2.225$ ppm). For coding of monosaccharide residues and complete structures, see Fig. 1. For comparison with M_8GP and M_9GP (possessing GlcNAc 2 in $\beta(1 \rightarrow 4)$linkage to GlcNAc 1), only the δ-values for the β-anomer of M_9G are given.

Reporter group	Residue	Chemical shift in		
		M_8GP	M_9GP	M_9G
H-1 of:	1	5.092	5.092	–
	2	4.608	4.608	4.714
NAc of:	1	2.007	2.007	–
	2	2.066	2.066	2.046
H-1 of:	3	≃4.77	≃4.77	4.772
	4	5.345	5.333	5.335
	4′	4.868	4.868	4.869
	A	5.401	5.401	5.407
	B	5.141	5.141	5.142
	C	5.059	5.308	5.308
	D_1	–	5.047	5.048
	D_2	5.059	5.059	5.063
	D_3	5.040	5.040	5.040
H-2 of:	3	4.228	4.228	4.229

cally shaped signal at $\delta = 5.345$ ppm in an intensity ratio 3 : 2, pointing to the presence of another carbohydrate structure in the mixture of glycopeptides. The signal at $\delta = 5.345$ ppm indicates strongly that the minor component of the mixture contains mannose 4 substituted by a terminal mannose C in $\alpha(1 \rightarrow 2)$-linkage. This can be derived from comparison of the oligosaccharides M_3G (mannose 4, δH-1 = 5.356 ppm) and M_4G (mannose 4, δH-1 = 5.343 ppm) from the urine of mannosidosis patients [4], showing that the attachment of mannose D_1 causes a small upfield shift for H-1 of mannose 4. In accordance with this interpretation, the H-1 signal of mannose C in M_9GP at $\delta = 5.308$ ppm is of equal intensity as that of mannose 4 at $\delta = 5.333$ ppm, while the H-1 signal of the terminal, $\alpha(1 \rightarrow 2)$-linked mannose C is found at $\delta \simeq 5.05$ ppm (see below). In other words, in the minor component at least mannose D_1 is missing, which is indeed reflected in the intensity ratio of the anomeric signals of mannose D_1, D_2 and D_3 at $\delta \simeq 5.05$ ppm, being different from 1 : 1 : 1. The anomeric signals of mannose A, B and 4′ each appear as single doublets in the spectrum of the mixture, with relative intensities of 1 : 1 : 1. The intensity of each of these doublets is equal to the sum of the intensities of the H-1 doublets of mannose 4. Consequently, the lower branches of the minor constituent are terminated by mannose D_2 and D_3, respectively. It can be concluded that the second component is a glycopeptide containing eight mannose residues (M_8GP), without mannose D_1 as compared to M_9GP.

The spectral region at $\delta \simeq 5.05$ ppm, comprising the anomeric signals of terminal $\alpha(1 \rightarrow 2)$-linked mannoses, is rather complex. For the unambiguous assignment of the signals, use is made, inter alia, of the relative intensities of the doublets (at $\delta =$ 5.059, 5.047 and 5.040 ppm), being 7 : 3 : 5. Based upon its relatively low intensity, the signal at $\delta =$ 5.047 ppm is ascribed to mannose D_1 in M_9GP. The signal at $\delta = 5.059$ ppm belongs partly to mannose D_2 in both M_8GP and M_9GP. This assignment can be deduced from the sensitivity of this chemical shift to the configuration of the anomeric centre of GlcNAc 2 in M_9G [4]. The anomeric signal of mannose C in M_8GP contributes to the intensity of the signal at $\delta = 5.059$ ppm (compare with M_3G and M_4G * [4]), thereby making this the most intense signal in this area. Finally, the doublet at $\delta = 5.040$ ppm is attributed to mannose D_3.

The relatively large number of *N*-acetyl signals in the spectrum ($2.1 < \delta < 2.0$ ppm) reflects a heterogeneity of the peptide moiety of M_8GP and M_9GP. This comes also to expression in the line-broadening of the anomeric signals of GlcNAc 1 and 2. As shown previously [2,6], no other structural reporter group signals are affected noticeably by heterogeneity of the peptide moiety.

The 500 MHz ^1H-NMR data reveal that this glycopeptide preparation from bovine lactotransferrin shows microheterogeneity in the carbohydrate part, with regard to the number of mannose residues.

The structures of the two main components are given in Fig. 1. The M_8GP oligomannoside-type structure represents a novel constituent. The analysis of this mixture of two closely related compounds shows that, also for oligomannoside-type structures, high-resolution ^{1}H-NMR spectroscopy is suited to describe microheterogeneity in terms of well-defined structures, which may ultimately lead to a further understanding of this phenomenon.

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References

1 Vliegenthart, J.F.G., Van Halbeek, H. and Dorland, L. (1981) Pure Appl. Chem. 53, 45–77
2 Van Halbeek, H., Dorland, L., Vliegenthart, J.F.G., Schmid, K., Montreuil, J., Fournet, B. and Hull, W.E. (1980) FEBS Lett. 114, 11–16
3 Van Halbeek, H., Dorland, L., Veldink, G.A., Vliegenthart, J.F.G., Michalski, J.-C., Montreuil, J., Strecker, G. and Hull, W.E. (1980) FEBS Lett. 121, 65–70
4 Van Halbeek, H., Dorland, L., Veldink, G.A., Vliegenthart, J.F.G., Strecker, G., Michalski, J.-C., Montreuil, J. and Hull, W.E. (1980) FEBS Lett. 121, 71–77
5 Chéron, A., Spik, G. and Montreuil, J. (1981) C. R. Acad. Sci. Paris, in the press
6 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
7 Montreuil, J. (1980) Adv. Carbohydr. Chem. Biochem. 37, 157–223