^{1}H-NMR analysis of the sugar structures of glycoproteins as their pyridylamino derivatives

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The ^{1}H-NMR spectra of a series of pyridylamino (PA-) derivatives of oligosaccharides were obtained and compared with those of the corresponding asparagine-linked sugar chains in order to elucidate the effect of the PA-group on the chemical shifts of structural-reporter signals. The effects were found to be localized within the two residues from the end group. Thus, the data for asparagine-linked chains in the literature are applicable to PA-derivatives, so the combination of pyridylamination and NMR measurements greatly reduces the time required for structure analysis of sugar chains of glycoproteins, because the isolation and purification of the chains as PA-derivatives are easy and efficient.

Sugar structure *Glycoprotein* *Oligosaccharide* *Pyridylamino derivative* *NMR* *Structural-reporter signal*

1. INTRODUCTION

Vliegenthart et al. [1] have shown that high resolution ^{1}H-NMR spectroscopy is a powerful tool for the elucidation of the chemical structures of asparagine-linked sugar chains isolated from glycoproteins. They used Asn-glycopeptides isolated from glycoproteins by means of enzymatic digestion and chromatography.

We have developed a fluorescence labeling method, in which the reducing ends of sugar chains are tagged with 2-aminopyridine [2]. The fluorescent pyridylamino (PA-) derivatives of sugar chains can be detected with high sensitivity and separated easily on a HPLC column owing to the hydrophobicity of the PA-group [3]. We performed NMR studies on several PA-derivatives of sugar chains derived from glycoproteins in an attempt to develop a simple and rapid way of determining the chemical structures of the sugar chains.

We studied the influence of the PA-group on the chemical shifts of structural-reporter groups to determine whether or not previously reported values for these shifts can be applied to PA-sugar chains.

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Abbreviations: R, GlcNAcβ1-4GlcNAc-PA; M1, Manβ1-4R; M2, Manα1-3Manβ1-4R; M3, Manα1-6-(Manα1-3)Manβ1-4R; M5, Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ1-4R; M6, Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-3)Manβ-4R; M9, Manα1-2Manα1 - 6(Manα1 - 2Manα1 - 3)Manα1 - 6(Manα1 - 2Manα1 - 2Manα1-3)Manβ1-4R; $Man_5GlcNAc_4$-PA, Manα1-6-(Manα1-3)Manα1-6(GlcNAcβ1-2Manα1-3) (GlcNAcβ1-4)Manβ1-4R; PA-biantennary sugar chain, Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)-Manβ1-4R; PA-triantennary sugar chain, Galβ1-4GlcNAcβ1 - 2Manα1 - 6(Galβ1 - 4GlcNAcβ1 - 2[Galβ1 - 4GlcNAcβ1-4]Manα1-3)Manβ1-4R; PA-, pyridylamino

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2. MATERIALS AND METHODS

2.1. *Materials*

α-Mannosidase (jack bean) was purchased from Sigma, St. Louis, and pronase from Seikagaku Kogyo, Tokyo. $Man_3GlcNAc_2$-Asn was isolated from riboflavin-binding protein (Japanese quail egg white) by means of pronase digestion in our laboratory. M5, M6 and $Man_5GlcNAc_4$-PA were obtained from ovalbumin by means of hydrazinolysis, *N*-acetylation and then pyridylamination according to our reported method [3]. In the same way, PA-biantennary and PA-triantennary sugar chains were obtained from α_1-acid glycoproteins donated by Dr K. Schmid (Boston University), and M9 from soybean agglutinin [4]. Preparation of M3 from Japanese quail ovomucoid was performed as reported [5], and PA-chitobiose was obtained from chitobiose by means of the reported method [3]. M1 was obtained through digestion of

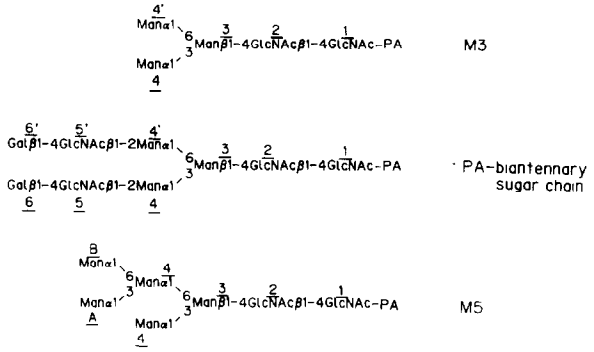

Fig.1. Code for monosaccharide residues in sugar chains.

M3 with α-mannosidase. M2 was obtained by partial acetolysis of M3 according to Kocourek and Ballou [6]. The PA-oligosaccharides thus obtained were purified by HPLC on a C18 reversed-phase column.

Table 1

^1H chemical shifts of structural-reporter groups of PA-oligosaccharides and $Man_3GlcNAc_2$-Asn

Reporter group	Residue	R	M1	M2	M3	$Man_3GlcNAc_2$-Asn	
						A	B
H-1	1	–	–	–	–	5.045	5.058
	2	4.641			4.623	4.613	4.616
			(4.668)	(4.665)	(4.639)		
	3	–			4.772	4.779	4.762
			(4.765)	(4.780)	(4.768)		
	4	–	–		5.106	5.100	5.107
				(5.125)	(5.114)		
	4′	–	–	–	4.915	4.914	4.907
					(4.915)		
H-2	3	–			4.243	4.254	4.226
			(4.055)	(4.215)	(4.232)		
	4	–	–		4.069	4.066	4.056
				(4.078)	(4.070)		
	4′	–	–	–	3.976	3.972	3.966
					(3.971)		
NAc	1	1.943			2.004	2.011	–
			(1.956)	(1.954)	(2.001)		
	2	2.067			2.068	2.073	–
			(2.064)	(2.061)	(2.067)		

The data are for 30°C. In parentheses, the data for 50°C are shown. The code for monosaccharide residues is shown in fig.1. (A) Data for 27°C, obtained in our laboratory; (B) reported data for 27°C [7]

Table 2

^{1}H chemical shifts of structural-reporter groups of PA-sugar chains

Reporter group	Residue	M5		PA-biantennary sugar chain	
H-1	1		5.073[a]		5.094[a]
			(5.068)		
	2	4.645	4.603		4.616
		(4.660)	(4.636)	(4.671)	
	3	4.784	4.8		4.765
		(4.778)	(4.772)	(4.763)	
	4	5.107	5.092	5.123	5.121
		(5.114)	(5.124)	(5.135)	
	4′	4.878	4.873	4.929	4.928
		(4.878)	(4.880)	(4.929)	
	A	5.102	5.092		
		(5.109)	(5.124)		
	B	4.913	4.908		
		(4.914)	(4.918)		
	5			4.584	4.582
	5′			4.584	4.582
	6			4.472	4.467
	6′			4.476	4.473
H-2	3	4.242	4.255	4.235	4.246
		(4.230)	(4.224)	(4.231)	
	4	4.083	4.074	4.192	4.190
		(4.081)	(4.074)	(4.193)	
	4′	4.152	4.145	4.113	4.109
		(4.142)	(4.131)	(4.110)	
	A	4.069	4.067		
		(4.069)	(4.074)		
	B	3.987	3.983		
		(3.987)	(3.981)		
NAc	1	1.949	2.010	1.945	2.004
		(1.951)	(2.021)	(1.956)	
	2	2.064	2.061	2.075	2.079
		(2.060)	(2.063)	(2.077)	
	5			2.055	2.050
				(2.059)	
	5′			2.048	2.046
				(2.053)	

[a] Data reported for glycopeptides having the same carbohydrate structures at 27°C, and those in parentheses, at 77°C [8]

The data are for 30°C. In parentheses, the data for 50°C are shown. The code for monosaccharide residues is shown in fig.1

2.2. *Methods*

^{1}H-NMR measurements at 500 MHz were performed with a JEOL GX-500s spectrometer. Samples were dissolved in 100% deuterated water obtained from Merck Sharp and Dohme, Canada. The homogated decoupling technique was used to suppress the peak of HDO, which was an isotopic contaminant present in the solvent. On measure-

ment at various temperatures, signals under the HDO peak were detected.

3. RESULTS AND DISCUSSION

We chose M3 as a representative PA-sugar chain for the assignment of the signals of structural-reporter groups, because Manα1-6(Manα1-3)Man-β1-4GlcNAcβ1-4GlcNAc is the common core structure of asparagine-linked sugar chains (see fig.1). We obtained ^{1}H-NMR spectra for R, M1, M2 and M3. For R, the resonance of the proton at position 1 (H-1) of GlcNAc-2 and those of the NAc of GlcNAc-1 and GlcNAc-2 could be assigned unambiguously, as shown in table 1. In the same way, additional reporter signals at 4.765 and 4.055 ppm for M1 were assigned to the H-1 and H-2 of Man-3, respectively. A new signal at 5.125 ppm for M2 was assigned to the H-1 of Man-4. This assignment was confirmed by measuring the coupling constants of the H-1 signals of Man-3 and Man-4 in M3, which were expected to be ~1.8 Hz for the α-linkage and 0.7–0.8 Hz for the β-linkage [1]. Glycopeptides were found to show downfield shifts of 0.16 ppm for the H-2 signal of Man-3, of which the 3-OH is replaced by an α-mannosyl residue [1]. Therefore, the H-2 signal of Man-3 in M2 can be assigned as shown in table 1. The reporter signals of M3 were assigned as shown in table 1 with reference to the data for R, M1 and M2. The chemical shifts of the structural-reporter groups of M3 are identical with those of $Man_3GlcNAc_2$-Asn, as shown in table 1, except for in the case of the H-1 of GlcNAc-1, which disappeared upon the pyridylamination. Table 2 shows that the influence of a PA-group on the chemical shifts is at least restricted to the GlcNAcβ1-4GlcNAc-PA residue. This was confirmed by obtaining the NMR spectra of PA-derivatives of high-mannose type sugar chains (M5, M6 and M9), a hybrid type sugar chain ($Man_5GlcNAc_4$-PA) and complex type sugar chains (PA-biantennary and PA-triantennary sugar chains) (see fig.1). Comparison of the data for PA-sugar chains with those for glycopeptides obtained through pronase digestion (table 2, two examples are shown) showed that each reporter signal undergoes the same chemical shift, except for the signals of the NAc of GlcNAc-1 and the H-1 of GlcNAc-2, showing that the reported values in the literature for the chemical shifts of structural-reporter groups of glycopeptides are applicable to PA-sugar chains when those of the core PA-chitobiose residues are taken into account.

Since the purification of sugar chains is rapid and simple after the reducing ends of the sugar chains have been pyridylaminated, the whole procedure can be completed within a week, including NMR spectral measurements.

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REFERENCES

[1] Vliegenthart, J.F.G., Dorland, L. and Van Halbeek, H. (1983) Adv. Carbohydr. Chem. Biochem. 41, 209–374.
[2] Hase, S., Ikenaka, T. and Matsushima, Y. (1978) Biochem. Biophys. Res. Commun. 85, 257–263.
[3] Hase, S., Ibuki, T. and Ikenaka, T. (1984) J. Biochem. 95, 197–203.
[4] Lis, H., Sharon, N. and Katchalski, E. (1966) J. Biol. Chem. 241, 684–689.
[5] Hase, S., Okawa, K. and Ikenaka, T. (1982) J. Biochem. 91, 735–737.
[6] Kocourek, J. and Ballou, C.E. (1969) J. Bacteriol. 100, 1175–1181.
[7] Nomoto, H., Endo, T. and Inoue, Y. (1982) Carbohydr. Res. 108, 91–101.
[8] Carver, J.P., Grey, A.A., Winnik, F.M., Hakimi, J., Ceccarini, C. and Atkinson, P.H. (1981) Biochemistry 20, 6600–6606.