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Application of High-Field Proton Magnetic Resonance Spectroscopy in the Structural Determination of Membrane-Derived Sindbis Virus Glycopeptides†

John Hakimi, Jeremy Carver, and Paul H. Atkinson*

ABSTRACT: Sindbis virus membrane glycopeptides have been purified in chemical quantities and their oligosaccharide structures analyzed by ^{1}H NMR spectroscopy at 360 MHz. Interpretable spectra could be obtained with approximately 100 μg of oligosaccharide. Spectral analysis of the sialyl glycopeptides S1, S2, and S3 at high and low temperatures confirms their structures to be NANAα(2,3)Galβ(1,4)-GlcNAcβ(1,2)Manα(1,6)-[NANAα(2,3)Galβ(1,4)-GlcNAcβ(1,2)Manα(1,3)]-Manβ(1,4)GlcNAcβ(1,4)-[Fucα-(1,6)]-GlcNAcβ1-Asn. These are heterogeneous with respect to sialic acid (NANA). Spectra of two *endo*-β-*N*-acetylglucosaminidase products of the S4 glycopeptides are reported. The interpretation of these spectra is consistent with $Man_5GlcNAc$ and $Man_7GlcNAc$ oligosaccharide structures. Their chemical shifts are essentially identical with those reported for ovalbumin glycopeptides of the same composition, with exception to the perturbations arising from their oligosaccharide nature.

Interest in the elucidation of oligosaccharide structures in membrane glycoproteins derives from accumulating evidence that the oligosaccharides of membrane glycoproteins may be involved in cell surface recognition events [reviewed by Frazier & Glaser (1979); Neufeld & Ashwell, 1980]. In addition, some studies suggest that glycosylation is required for the establishment and preservation of specific protein conformation (Gibson et al., 1981), which in turn affects the biological properties of some glycoproteins (Kaluza et al., 1980). In the latter studies, a quite subtle alteration in glycosylation changed antigenic characteristics of Semliki Forest virus intracellular proteins p62 and E1. Presumably such alterations might include changes in the ratio of higher mannose polymers to lower mannose polymers previously described in the four major Sindbis virus (SbV) S4 glycopeptides of glycoproteins E1 and E2 (Hakimi & Atkinson, 1980a).

To better detect fine structural details, we have used high-field ^{1}H NMR[1] analysis of membrane glycopeptides. Such spectroscopy at 360 MHz is a powerful tool in structural determination of oligosaccharides and glycopeptides [for review, see Carver & Grey (1981); Montreuil, 1980]. Unlike classical methods of oligosaccharide structural analysis, ^{1}H NMR spectroscopy is a nondestructive technique which yields information on sugar composition, anomeric configuration, linkage, number of antennae, some sequential details, and tertiary structure of oligosaccharides. It also can be used to assay sample homogeneity during purification (Cohen & Ballou, 1980; Atkinson et al., 1981) which is again illustrated in this report, because mixtures give rise to spectra with resonances at nonintegral ratios of intensity. Considerable ^{1}H NMR spectra of sialyl-type glycopeptides have been published (Dorland et al., 1978; Fournet et al., 1978; Narasimhan et al., 1980; Vliegenthart et al., 1981), and chemical shift assignments have been established. Such detailed analysis on the oligomannosidic-type glycopeptides has also been reported (Gorin et al., 1969; Cohen & Ballou, 1980; Carver et al., 1981;

† From the Department of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York 10461 (J.H. and P.H.A.), and the Department of Medical Genetics and Toronto Biomedical NMR Centre, University of Toronto, Toronto, Ontario, Canada M5S 1A8 (J.C.). *Received June 10, 1981.* This work was supported by grants from the National Institutes of Health (CA13402 and CA13330) and the Medical Research Council of Canada (MT-3732 and MA-6499), the National Cancer Institute of Canada. J.H. is a Postdoctoral Fellow of the Leukemia Society of America. P.H.A. was an Established Investigator of the American Heart Association during the course of this work.

[1] Abbreviations used: ^{1}H NMR, proton magnetic resonance; Man, mannose; Gal, galactose; Fuc, fucose; GlcNAc, *N*-acetylglucosamine.

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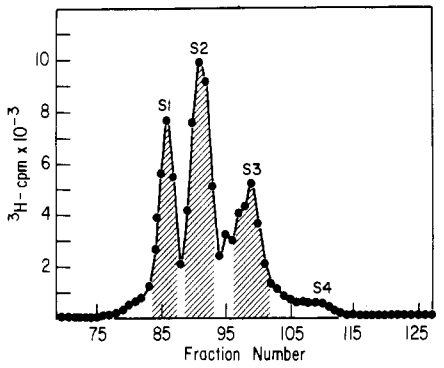


FIGURE 1: Bio-Gel P6 chromatography of complex-type [^3H]Man SbV glycopeptides. SbV glycopeptides were fractionated on Bio-Gel P6 (200–400 mesh, 0.9 × 150 cm). Pooled glycopeptides were redigested with Pronase and applied to a second Bio-Gel P6 column (–400 mesh, 0.9 × 175 cm); 0.50-mL fractions were collected. Elution of glycopeptides was monitored by assaying 5-μL aliquots for radioactivity in 10 mL of Aquasol-2 (New England Nuclear).

Carver & Grey, 1981; Van Halbeek et al., 1980a,b). In this paper, these published chemical shift assignments have been used to deduce membrane-derived glycopeptide structures.

We have isolated membrane glycopeptides in chemical quantities from purified SbV for structural analysis by ^{1}H NMR spectroscopy at 360 MHz. The structures of S1, S2, and S3 glycopeptides and the average structure of the heterogeneous mixture of S4 glycopeptides from SbV have been reported (Burke, 1976; Keegstra et al., 1975; Burke & Keegstra, 1979). We have obtained ^{1}H NMR spectra for S1, S2, and S3 glycopeptides, and our assignments are in agreement with the published structures. Two of the four high mannosyl oligosaccharides from the S4 glycopeptide mixture have been purified, and their structures have been elucidated by using ^{1}H NMR spectroscopy. This work in part has been previously presented (Hakimi & Atkinson, 1980b). A recent report has demonstrated the applicability of ^{1}H NMR spectroscopy coupled with methylation analysis in structural studies of mixtures of oligosaccharides from plasma membrane glycoproteins (Debray et al., 1981). Our report demonstrates the applicability of ^{1}H NMR alone as an assay in purification and a method of structural determination of sialyl and oligomannosyl glycopeptides from defined membrane glycoproteins. Our approach has been to use the chemical shifts of C2-H in combination with those of C1-H at two different probe temperatures in the determination of these structures [see Carver & Grey (1981)]. As in our previous study (Carver et al., 1981), the chemical shifts of resonances arising from many hydrogens were markedly temperature dependent. We show in addition that the method is quite sensitive because interpretable spectra could be obtained from as little as 100 μg of oligosaccharide.

Materials and Methods

Purified SbV was prepared from chicken embryo fibroblasts as previously described (Hakimi & Atkinson, 1980a). Seven separate purified virus preparations each harvested from approximately 50 roller bottles were pooled (unless otherwise indicated). Purified SbV labeled with [2-^{3}H]mannose (New England Nuclear; 2 Ci/mmol) was added to unlabeled virus (Hakimi & Atkinson, 1980a). Glycopeptides were prepared by exhaustive Pronase (Calbiochem) digestion, desalted on Sephadex G-10 (Pharmacia), and fractionated on Bio-Gel P6 (Bio-Rad) as described previously (Hakimi & Atkinson, 1980a). The complex-type glycopeptides were rechromatographed on Bio-Gel P6 (–400 mesh, 0.9 × 175 cm) eluted with 0.10 M NH_4HCO_3. This separation of S1, S2, and S3 is shown in Figure 1, and hatched areas indicate pooled fractions. The S4 glycopeptides were digested with endo-β-*N*-acetylglucosaminidase-H prepared by the method of Tarentino & Maley (1974). The oligosaccharides were passed through an AG-50X2 ion-exchange column (200–400 mesh, 0.9 × 21 cm) (Bio-Rad) equilibrated in sodium acetate buffer, pH 2.6, as previously described (Huang et al., 1970). This step was used to remove GlcNAc-Asn-X and incompletely digested peptides. The oligosaccharides were fractionated on Bio-Gel P2 (–400 mesh, 0.9 × 175 cm) eluted with 1 mM sodium azide. The individual oligosaccharides were rechromatographed on the same column. The glycopeptides and oligosaccharides were desalted on Sephadex G-10 (2.5 × 120 cm) eluted with deionized water.

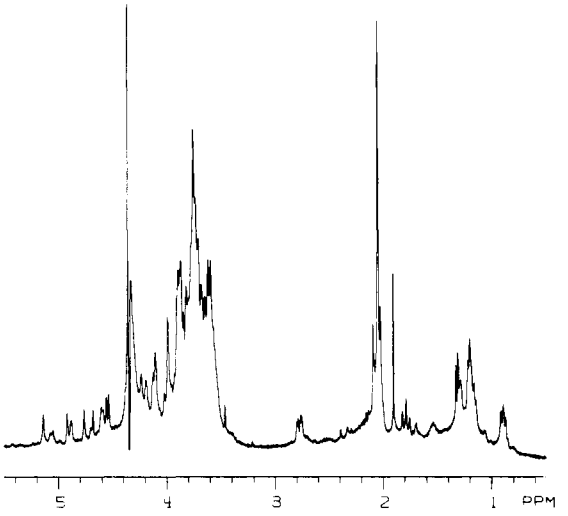


FIGURE 2: ^{1}H NMR spectrum of S1 glycopeptide at 70 °C. Suppression of the HDO signal has been employed. This spectrum was accumulated from 7168 scans.

The purified glycopeptides and oligosaccharides were prepared for ^{1}H NMR analysis by chromatography on Chelex-100 (Bio-Rad, 200–400 mesh, 0.9 × 10 cm, Na^+ form) to remove divalent cations. Glycopeptides and oligosaccharides were pooled, lyophilized, exchanged twice in deuterium oxide (Merck Sharp & Dohme, 99.7 atom % D), and dried over P_2O_5 in vacuo for several days. Prior to analysis, samples were redissolved in 100 μL of deuterium oxide (Stohler Isotope Chemicals, Waltham, MA, 100.0 atom % D). Acetone was added to samples at roughly equimolar concentration as an internal standard for chemical shifts. Microcells, of 100 μL (Wilmad Co., 529-E) were used. Spectral analysis was performed on a Nicolet 360-MHz spectrometer at the Toronto Biomedical NMR Center operating in the Fourier transform mode at probe temperatures of 23 and 70 °C. Instrument conditions and parameters were as previously described (Atkinson et al., 1981).

Results

The complete 360-MHz ^{1}H NMR spectra of SbV glycopeptide S1 at 70 °C is shown in Figure 2. An expansion of the anomeric regions in the spectra of the S1, S2, and S3 glycopeptides at 70 °C is compared in Figure 3. The chemical shifts of the anomeric GlcNAc, Gal, mannose C1-H and C2-H, fucose C1-H, C5-H, and C6-H, and sialic acid C3-H axial and equatorial proton resonances from these three glycopeptides at 23 and 70 °C are compiled in Table I. The 8–10-Hz doublets in the spectrum of S1 at 5.053, 4.683, and 4.59 ppm arise from C1 protons of β-linked *N*-acetylglucosamine (GlcNAc) residues (Dorland et al., 1977a,b, 1978). The resonance at 5.053 ppm is characteristic of GlcNAc attached

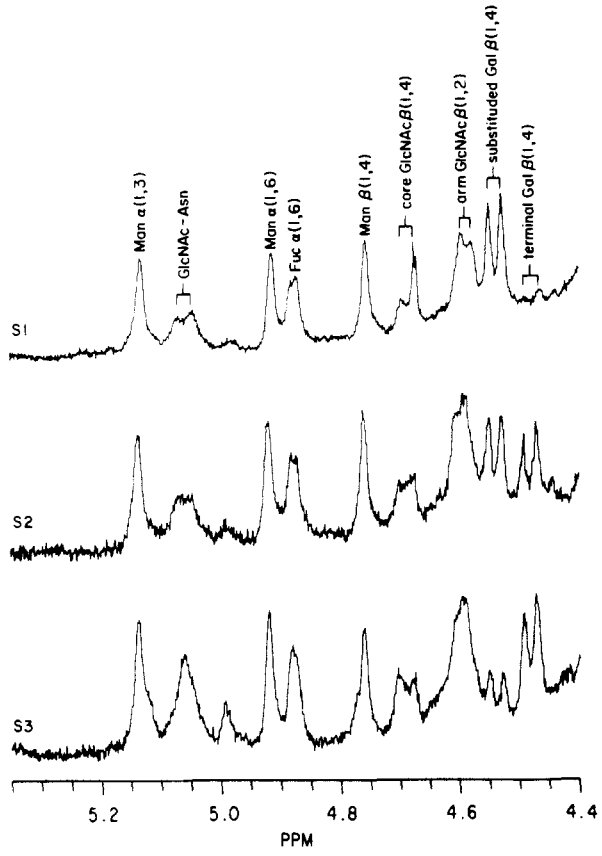


FIGURE 3: Expansion of the anomeric region of the ^{1}H NMR spectrum of SbV complex-type glycopeptides at 70 °C. The spectra of S1, S2, and S3 were accumulated from 7168, 1600, and 9216 scans, respectively.

to asparagine. Resonances characteristic of asparagine (β-CH_2 protons) are obscured by equatorial C3 hydrogens of sialic acid at 2.77 ppm (Figure 2). The resonance at 4.585 ppm belongs to C1 protons of the GlcNAc residues substituting the two branching mannose (Man) residues (see scheme in Table I). At this temperature, the chemical shifts of C1 protons of these latter GlcNAc units are indistinguishable from each other regardless of whether they are attached to α(1,6)- or α-(1,3)-linked mannose. The resonances at 5.129 and 4.910 ppm are characteristic of the C1 protons of substituted α(1,3)- and α(1,6)-linked mannose residues respectively. The resonance appearing at 4.757 ppm is characteristic of the C1 proton of a disubstituted anomeric β(1,4)-Man. These assignments are confirmed by analysis of mannosyl C1-H and C2-H resonances at 23 °C (Table I) as described previously (Carver & Grey, 1981; Dorland et al., 1977a). The resonances at the above-mentioned chemical shifts for GlcNAc and Man are unchanged in all three spectra, indicating similar positions and linkages of these monosaccharides.

The two 8-Hz coupled resonances in the spectrum of S2 appearing at 4.536 and 4.480 ppm are characteristic of anomeric β-linked galactose (Gal) units (Dorland et al., 1978). These two resonances have different intensities in the three glycopeptides. The resonance at 4.536 ppm in the spectrum of S1 glycopeptide is indicative of two β-linked Gal residues substituted by an α(2,3)-linked sialic acid. This α(2,3)-sialic acid substitution of Gal results in a characteristic downfield shift of 0.11 ppm in the resonance of the C1 proton in relation to an unsubstituted galactose residue (Dorland et al., 1978). The spectrum of the S2 glycopeptide reveals one Gal residue substituted by an α(2,3)-sialic acid and one unsubstituted β(1,4)-Gal residue whose C1 proton resonates at 4.480 ppm.

Table I: Chemical Shifts (δ) of Complex-Type Sindbis Glycopeptide Protons Derived from High-Resolution Proton NMR Spectra at 360 MHz

SAα(2,3)-Galβ(1,4)-GlcNAcβ(1,2)-Manα(1,6)
Fucα(1,6)
Manβ(1,4)-GlcNAcβ(1,4)-GlcNAcβ1-Asn
SAα(2,3)-Galβ(1,4)-GlcNAcβ(1,2)-Manα(1,3)

	temp (°C)	δ of C1-H residues (ppm)							δ of C2-H residues (ppm)				δ of C3-H of sialic acid (ppm)		δ of fucose protons (ppm)		
		1	2	3	4	4′	5	5′	6	6′	3	4	4′	eq	ax	C5	C6	C1
S1	70	5.053	4.683	4.755	4.910	5.129	4.585	4.585	4.536	4.536	4.224	4.179	4.114	2.766	1.783	4.092	*c*	4.872
	23	5.056	*a*	*a*	4.925	5.117	4.576	4.576	4.550	4.550	4.254	4.128	4.194	2.761	1.802	4.112	1.201	*a*
S2	70	5.059	4.687	4.757	4.915	5.135	4.596[b]	4.596[b]	4.536	4.480	4.226	4.184	4.116	2.772	1.784	4.096	*c*	4.874
	23	5.055	*a*	*a*	4.924	5.118	4.579[b]	4.579[b]	4.548	4.472	4.248	4.129	4.192	2.759	1.802	4.112	1.201	*a*
S3	70	5.056	4.687	4.757	4.916	5.134	4.592[b]	4.592[b]	4.481	4.481	4.223	4.182	4.121	2.768	1.781	4.098	*c*	4.876
	23	5.055	*a*	*a*	4.926	5.119	4.581[b]	4.581[b]	4.471	4.471	4.251	4.133	4.192	2.755	1.796	4.112	*c*	*a*

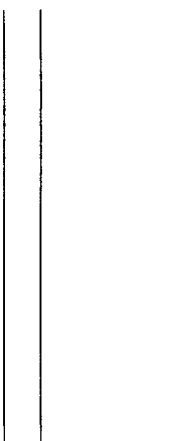
[a] Obscured by residual HDO. [b] Center of unresolved peak. [c] Obscured by amino acid peaks.

Unlike similar structures containing α(2,6)-linked sialic acid linkages, α(2,3)-sialic acid residues do not produce characteristic changes in the chemical shift of GlcNAc and Man anomeric protons (Dorland et al., 1978). The location of the single sialic acid residue on the Manα(1,6) or the Manα(1,3) arm (scheme in Table I) can theoretically be determined by nuclear Overhauser enhancement experiments [see Carver et al. (1981)]. It is possible that S2 is a mixture of two glycopeptides where sialic acid attachment occurs on either branching arm. This may arise from the fact that these glycopeptides are derived from two glycoproteins E1 and E2. The spectrum of S3 glycopeptide contains predominantly unsubstituted β(1,4)-Gal residues, but the resonance at ~4.54 ppm which appears with fractional intensity indicates an approximately 40% contamination of this sample with a glycopeptide containing an α(2,3)-sialic acid linked to Gal, probably from S2 glycopeptide. Likewise, the intensities of the resonances characteristic of substituted and unsubstituted β-Gal residues in the S2 glycopeptide spectrum are not equal; a 50% contamination of this sample with S1 glycopeptide can be estimated. This contamination is further verified by the appearance of subintegral intensities of resonances at ~2.76 and ~1.79 ppm (data not shown) characteristic of α(2,3)-linked sialic acid C3-H chemical shifts for equatorial and axial isomers (Dorland et al., 1978). This illustrates that the separation of glycopeptides on Bio-Gel P6 (Figure 1) as pooled was inadequate for obtaining homogeneous glycopeptides. *N*-Acetyl residues of GlcNAc and sialic acid found at the region of ~2.0 ppm in these spectra also supply structural information as reported by Dorland et al. (1978). Fucose can be identified as α(1,6) in all three glycopeptides by the characteristic resonances at chemical shifts of 4.87, ~4.1, and 1.20 ppm produced by the C1, C5, and C6 protons, respectively (Dorland et al., 1977b; Strecker et al., 1978). Fucose linkage to the core GlcNAc (attached to asparagine) can be confirmed by the chemical shift of the adjacent β(1,4)-linked GlcNAc. In S1, S2, S3, and other similar compounds, the β(1,4)-GlcNAc has a characteristic chemical shift at ~4.69 ppm. In asialiotransferrin which contains no fucose, the β(1,4)-GlcNAc has a chemical shift at ~4.61 ppm (Dorland et al., 1977a).

There are several unidentified resonances which may be attributed to amino acid protons other than Asn in the peptide portion of these compounds (Figures 1 and 2). The glycopeptides are derived from two proteins with one or two glycosylation sites, and the sequence surrounding any glycosylated asparagine may be different. Differential Pronase digestion of the protein backbone surrounding asparagine can also contribute to this amino acid heterogeneity.

The ^{1}H NMR spectra of only two S4 mannosyl oligosaccharides could be obtained (Figure 4). Only one-third the amount of virus used for preparing S1, S2, and S3 glycopeptides was available. This resulted in poorer signal to noise ratio explaining the observed lack of resolution compared to the sialyl complex type glycopeptides (Figure 3). The anomeric proton region of these spectra are shown in Figure 4, and chemical shifts are listed in Table II. S4C and S4E correspond in molecular weight to $Man_7GlcNAc$ and $Man_5GlcNAc$, respectively (Hakimi & Atkinson, 1980a). The resonance appearing at 5.25 ppm and the shoulder at 4.76 ppm are the C1 protons of the α and β anomers of the reducing GlcNAc residue, respectively. These resonances are not well resolved since small quantities of these oligosaccharides were available for analysis. The resonance at 4.783 ppm is characteristic of the C1 proton of β-Man-linked residues, and the large intensity at 5.129 ppm corresponds to C1 protons of two

Table II: Chemical Shifts (δ) of Sindbis Virus Derived Mannosyl Oligosaccharides from High-Resolution ^{1}H NMR Spectra at 360 MHz



compd	temp (°C)	δ of C1-H residues (ppm)									δ of C2-H residues							
		1	2	3	4	5	6	7	8	9	3	4	5	6	7	8	9
S4E	70	*b*	*b*	4.783	4.888	5.129	4.923	5.129	*b*	*b*	*b*	*b*	4.083	3.987	4.083	*b*	*b*
	23	5.245	*a*	*a*	*a*	5.102	*a*	5.102	*a*	*a*	4.269	4.153	4.077	3.986	4.077	*b*	*b*
S4C	70	*b*	*b*	4.773	4.884	5.341	5.117	5.118	5.064	5.064	*a*	*a*	*a*	4.028	4.074	4.074	4.074
	23	5.245	*a*	*a*	*a*	5.353	5.150	5.105, 5.072[c]	5.055	5.041	4.244	4.147	4.114	4.023	4.070	4.070	4.070

[a] Obscured by residual HDO. [b] Not determined. [c] Two resonances arise from residue number 7 in reducing terminal oligosaccharides, from the effect on this α3t anomeric hydrogen of the α and β anomeric configurations of the Man,GlcNAc oligosaccharide [for further discussion of this effect, see Cohen & Ballou (1980); Carver et al., 1981]. [d] Acetate reference at 1.900 ppm at 70 °C and 1.910 at 23 °C.

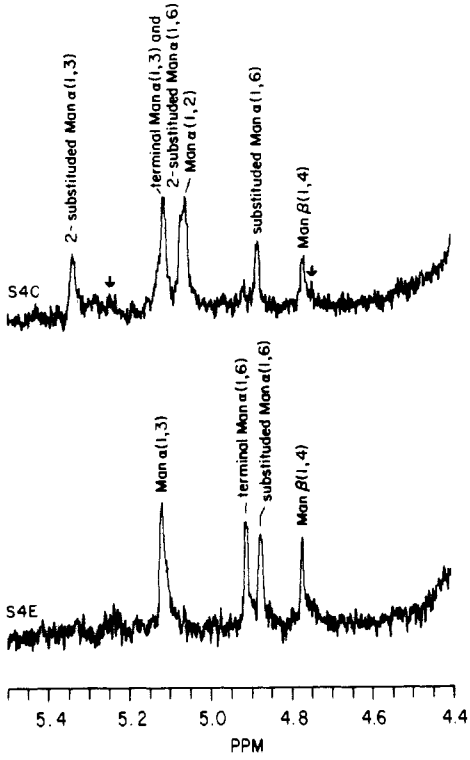


FIGURE 4: Expansion of the anomeric region of the ^{1}H NMR spectrum of SbV mannosyl oligosaccharides at 70 °C. The spectrum of S4C was accumulated from 512 scans and that of S4E from 256 scans. Arrows from left to right indicate the positions of the resonances of the α and β anomers, respectively, of the reducing GlcNAc residue.

terminal α(1,3)-mannose-linked residues. The resonances appearing at 4.888 and 4.923 ppm corresponded to C1 protons of disubstituted and terminal α(1,6)-linked Man residues, respectively.

The spectrum of S4E is identical with the ^{1}H NMR spectra of the oligosaccharide obtained from the ovalbumin glycopeptide $Man_5GlcNAc_2Asn$ at 360 MHz (Carver et al., 1981). The spectrum at 180 MHz of the same ovalbumin glycopeptide supports this assignment (Cohen & Ballou, 1980). In addition, the same structure has been deduced from a 500-MHz spectrum of a glycopeptide derived from urine whose chemical shifts are essentially the same as those reported for the ovalbumin glycopeptide (Van Halbeek et al., 1980a).

In the spectrum of S4C, the C1 protons of β(1,4)-linked and the disubstituted α(1,6)-linked mannose residues have resonances at chemical shifts similar to those observed in the S4E spectrum (Figure 4). The resonance at 5.341 ppm can be assigned to the C1 proton of an α(1,3)-linked Man unit substituted at its C2 position by another Man residue. The large downfield shift of the C1 proton of mannose 2-*O*-substituted with mannose is the "deshielding" effect described by Cohen & Ballou (1980). A carbohydrate contaminant is apparent in the S4C spectrum by the small resonance intensity at 4.923 ppm which is characteristic of a terminally linked α(1,6)-Man residue. Between 5.05 and 5.15 ppm, two large double intensity resonances are apparent. One can assign four monosaccharide residues in this region by using data from Cohen & Ballou (1980), Gorin et al. (1969), and Carver & Grey (1981) and the C2 proton data (see Table II). The double intensity resonance at 5.117 ppm corresponds to a terminally α(1,3)-Man and a C2-substituted Manα(1,6) residue. The resonance at 5.064 ppm corresponds to two terminally linked α(1,2)-Man residues. One is attached to an α(1,6)-Man residue and the second to an α(1,3)-Man residue. On the basis of ^{1}H NMR data of two IgM glycopeptide isomers (rGP-563II), one can distinguish which α(1,3)-Man residue is substituted by a terminal α(1,2)-Man (Cohen & Ballou, 1980). In addition, the C1 proton resonances of S4C are similar to the chemical shifts reported for ovalbumin glycopeptide $Man_7GlcNAc_2Asn$ (Cohen & Ballou, 1980; Atkinson et al., 1981).

Discussion

The use of high-field ^{1}H NMR demonstrates that this method of oligosaccharide structural analysis is applicable to glycopeptides of integral membrane glycoproteins. The oligosaccharide structures of the SbV glycopeptides S1, S2, and S3 discernible in the ^{1}H NMR spectra shown here are in agreement with those structures reported previously (Burke & Keegstra, 1979). ^{1}H NMR analysis confirms the structures of the S4 mannosyl oligosaccharides S4C and S4E to be $Man_7GlcNAc$ and $Man_5GlcNAc$ as previously reported (Hakimi & Atkinson, 1980a). By ^{1}H NMR analysis, the S4 structures are similar to those reported for ovalbumin glycopeptides of the same composition and are in agreement with the oligosaccharide processing intermediates originally reported (Hunt et al., 1978; Robbins et al., 1977; Tabas et al., 1978). One can also conclude from these spectra that the S1, S2, S3, and S4 structures of E1 and E2 glycoproteins contain essentially identical oligosaccharides, since these spectra were obtained from mixtures of glycopeptides derived from intact virus.

Though greater initial quantities of glycopeptide (approximately 50–100 nmol in 100 μL) are required for these studies, certain advantages over classical carbohydrate analysis are apparent. Both approaches result in similar structural assignments. The ^{1}H NMR method is unique in that it is capable of assaying oligosaccharide homogeneity simultaneously with structural information in a nondestructive manner. Therefore, essentially the entire carbohydrate sample is preserved for either further purification steps, additional structural characterization, or biological studies.

Acknowledgments

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Structure and Conformation of Pseudouridine Analogues†

Robert L. Lipnick,‡ John D. Fissekis,* and James P. O'Brien

ABSTRACT: The structural and conformational features of the "anomeric" DL-*trans*- and DL-*cis*-5-(3-hydroxytetrahydrofuran-2-yl)uracils (**3a**, **4a**) and five similar analogues were studied in order to determine their applicability as models of β- and α-pseudouridine. The 270-MHz proton NMR spectra were measured for all analogues to define their ring geometries in solution and to estimate the solution population of *model* N, S conformers in a two-state dynamic equilibrium treatment. Two sets of calculations were employed to evaluate the relative contributions of these states to the observed vicinal coupling constants related to the C(3′)–C(4′) fragment. In the first, similar geometries were assumed for each pair of conformers, while in the second, limited to **3**, the geometries were those derived from X-ray crystallographic data; both gave comparable results. The cis analogues **4a** and **4b** are excellent conformational models for α-pseudouridine. In the trans series (**3a–c**), the equilibrium is weighted toward the N conformer (~80%), differing from that found in β-pseudouridine for which each model conformer is equally populated. Possible implications of the conformational effects upon the pairing properties of pseudouridine in tRNA are discussed.

Pseudouridine (ψ, **1**; Figure 1), an isostere of uridine (U), is unique among the numerous naturally occurring pyrimidine or purine nucleosides in being the only C-nucleoside constituent of the nucleic acids (RNA) of both procaryotes and eucaryotes. It has been found as a component of the "constant" tetranucleotide TψCG(A)[1] in loop IV of almost every transfer RNA (tRNA) that is active in the elongation step of protein biosynthesis (Zamir et al., 1965; Sprinzl et al., 1978). In addition, two vicinal ψ residues have been identified at the 3′ end of the anticodon loop in certain tRNAs. The sequences ψψ or ψGψ, which are derived from UU or UGU, respectively, through site-specific enzymatic transitions at the macromolecular level, are required for the extended function of such tRNAs in gene repression (Turnbough et al., 1979; Bossi & Cortese, 1977; Cortese et al., 1974a,b; Singer et al., 1972; Allaudeen et al., 1972). The presence of ψ instead of U at these specific sites indicates that certain physicochemical properties unique to the C-nucleoside structure of ψ are critical to the biological function of tRNA molecules. Two features of ψ have been considered as possible contributors to its role in tRNA: (i) its potential to hydrogen bond with adenosine (A) via either the N(1),C(2) or the N(3),C(2) sites (Hurd & Reid, 1977) and (ii) its ability to form a covalent adduct with cysteine at the allylic C(1′) position (Lipnick & Fissekis, 1977).

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* Address correspondence to this author at the Division of Natural Sciences, State University of New York, College at Purchase, Purchase, NY 10577.

‡ Present address: U.S. Environmental Protection Agency, Office of Toxic Substances, Health and Environmental Review Division, Washington, D.C. 20460.

[1] Abbreviations used: ψ, β-pseudouridine; A, adenosine; G, guanosine; U, uridine; T, thymidine; C, cytidine; tRNA, transfer RNA; NOE, nuclear Overhauser effect; CNDO, complete neglect of differential overlap; DSS, sodium 4,4-dimethyl-4-silapentane-1-sulfonate.

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