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Note

^{13}C-N.m.r.-spectral study of two O-D-galactosylated tripeptides

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A series of studies have been published dealing with carbon-13 nuclear magnetic resonance (^{13}C-n.m.r.) data for some glycosylated amino acids, such as 4-*N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine[1], 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-serine[1], 3-*O*-α-D-mannopyranosyl-L-serine[2], 3-*O*-α-D-mannopyranosyl-L-threonine[2], 4-α- and -β-L-arabinofuranosyloxy-L-proline[2], 3-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine[3], 3-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonine[3], 3-*O*-α,β-D-galactopyranosyl-L-serine[3], 3-*O*-α,β-D-galactopyranosyl-L-threonine[3], 3-*O*-α- and -β-D-xylopyranosyl-L-serine[4], and 3-*O*-α- and β-D-xylopyranosyl-L-threonine[4]. In this work, it was shown that these simple compounds may be used as models (for ^{13}C-n.m.r.-spectral data) for the attachment of complex carbohydrates to proteins. This was postulated on the basis that the chemical shifts of the carbohydrate carbon atoms show very little pH-dependence (≤ 0.5) when the form of the amino acid is varied from the cationic to the anionic[1–4]. Study of these simple model compounds also showed that they may be used to detect possible carbohydrate interactions[3] that have recently been shown to exist in glycoproteins[5].

In continuation of this work, an article dealing with a ^{13}C-n.m.r. study of mono- and di-*O*-D-galactosylated dipeptides[6] has recently been published. In these series of model compounds, it was found that structural perturbations due to *O*-glycosylation of the dipeptides may exist.

We now present some ^{13}C-n.m.r. data for two *O*-α-D-galactosylated tripeptides (composed of Thr and Gly; see **1** and **3**) and for the nonglycosylated tripeptides (**2** and **4**). These contain the first ^{13}C-chemical shift data for a simple glycopeptide for which the carbohydrate carbon atoms are not influenced in the least by a neigh-

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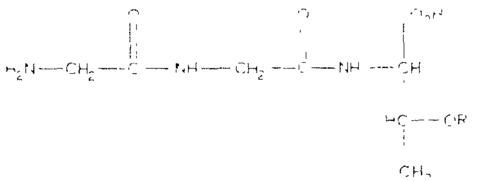
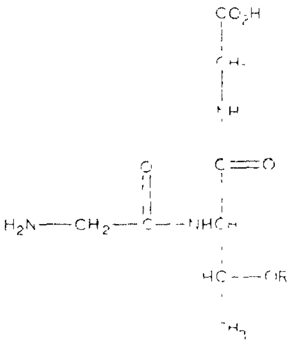
boring, charged species. Because of the types of model compounds studied, it proved feasible to compare the effects that glycosylation may have on the chemical shifts of the tripeptide.



1 R = α-D-Gal*p*
2 R = H



3 R = α-D-Gal*p*
4 R = H

EXPERIMENTAL

Materials and methods. — The synthesis of the *O*-α-D-galactosylated tripeptides **1** and **3** (see Table I) composed of L-Thr and Gly, as well as of their respective unglycosylated forms (**2** and **4**) has been published[7]. Samples for n.m.r. spectroscopy were prepared by dissolving the peptide or glycopeptide in deionized, distilled water. Adjustments of pH were made with M NaOH, or M HCl, using a Radiometer PHM63, digital pH-meter.

^{13}C-N.m.r. spectra were recorded with a JEOL-FX90Q instrument operating at 22.5 MHz (2.1 T) in the F.t. mode, by use of quadrature detection. Samples (1.5 mL) were contained in a 10-mm tube having a concentrically inserted, 5-mm tube containing D_2O to serve as the field-frequency lock. The probe temperature was maintained at 25° for all samples. For ^{13}C excitation, 90° radiofrequency pulses of 18 μs were used, and the carrier frequency was set 90 p.p.m. downfield from the ^{13}C resonance of Me_4Si. Time-domain data were collected in 8192 addresses. A spectral window of 5.5 kHz was used for recording the spectra. Fully proton-decoupled spectra were obtained when the noise-modulated, ^{1}H irradiation (having a bandwidth of 1.0 kHz) was centered 4 p.p.m. downfield from Me_4Si

TABLE I

^{13}C-N.M.R. CHEMICAL-SHIFT DATA[a] FOR NON- AND MONO-*O*-D-GALACTOSYLATED TRIPEPTIDES

Carbon atom	*Compound*			
	1[b]	**2**[c]	**3**[d]	**4**[e]
α1	100.3		100.8	
α2	69.9		69.8	
α3 α4	70.8		70.8	
α5	72.4		72.6	
α6	62.6		62.7	
Gly C-2 (N-terminal)	42.1	42.3	42.5	42.7
Gly C-2 (internal)	43.8	43.9		
Gly C-2 (C-terminal)			44.9	44.6
Thr C-3	76.9	69.3	75.9	68.2
Thr C-2	60.3	61.7	59.1	60.3
Thr C-4	19.6	20.5	19.0	19.8

[a]Chemical shifts for these compounds are given at neutral pH (5.8 → 7.2). Estimated precision for the chemical shifts is $\mp$0.05 p.p.m. A recycle time of 1 s was used for recording the spectra. [b]62mM in H_2O, pH 6.53; 38,651 accumulations. [c]50mM in H_2O, pH 7.15; 20,164 accumulations. [d]66mM in H_2O, pH 6.68; 22,214 accumulations. [e]95mM in H_2O, pH 5.83; 44,658 accumulations.

Chemical shifts are given relative to a trace of internal 1,4-dioxane (added only when chemical shifts were determined), whose chemical shift was taken to be 67.86 p.p.m. downfield from Me_4Si.

RESULTS AND DISCUSSION

Fig. 1A, 1B, 1C, and 1D, respectively, show the aliphatic region of the proton-decoupled, natural abundance, ^{13}C-n.m.r. spectra of the model compounds **1** (Gly-Gly-Thr*), **2** (Gly-Gly-Thr), **3** (Gly-Thr*-Gly), and **4** (Gly-Thr-Gly), where the asterisk indicates the point of attachment of an α-D-Gal*p* group. The chemical shifts of the various resonances, as well as the specific assignments of C-2 of Gly to specific glycine residues, are given in Table I.

Assignments of some of the resonances to specific carbon atoms of the carbohydrate and the amino acids were based on the results of previous work[3,6]; C-2 of glycine could only be assigned on the basis of the pH behavior. The C-2 atom of the internal glycine residue showed no titration behavior (see Table II). The C-2 atom of C-terminal glycine exhibited pH-dependence at low pH (<7.0), whereas the C-2 atom of N-terminal glycine exhibited pH-dependence at high pH (>7.0).

First, the ^{13}C-chemical-shift data for the amino acid residues of the glycosylated and nonglycosylated tripeptides may be compared (see Table III). On comparing compounds **1** and **2**, and compounds **3** and **4**, a general trend in the shift is observed. For the L-threonine residue of the various compounds, glycosylation produces, as

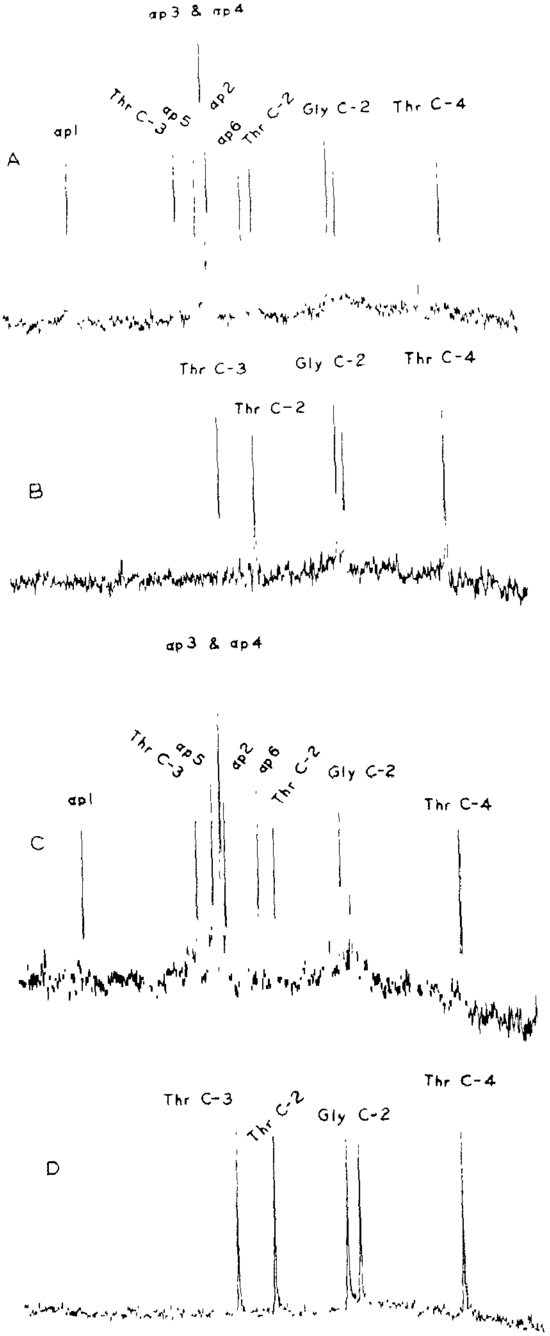


Fig. 1. The aliphatic carbon region (10-110 p.p.m.) of the proton-decoupled, natural-abundance, ^{13}C-n.m.r. spectra of model compounds **1** to **4**. [The exact spectral conditions for each model compound are given in Table I. αp refers to the α anomer of the pyranosyl group. (A) Model compound **1**, (B) model compound **2**; (C) model compound **3**, and (D) model compound **4**.]

TABLE II

CHEMICAL-SHIFT DIFFERENCE FOR THE CARBON ATOMS OF THE MODEL COMPOUNDS IN ACIDIC AND BASIC MEDIA[a]

Carbon atom	*Chemical-shift difference (δA − B)*			
	1	**2**	**3**	**4**
α1	+0.5		−0.2	
α2	−0.1		+0.1	
α3	−0.1		+0.1	
α4	−0.2			
α5	0.0		−0.2	
α6	0.0		0.0	
Gly C-2 (N-terminal)	−3.2	−3.2	−3.1	−3.1
Gly C-2 (internal)	−0.2	−0.1		
Gly C-2 (C-terminal)			−2.3	−1.9
Thr C-2	−2.0	−2.2	−0.1	+0.4
Thr C-3	−0.8	−0.8	−0.3	−0.1
Thr C-4	−0.2	−0.3	−0.2	0.0

[a]Chemical shift in acidic media is defined as: at pH $<$ 2.0. The chemical shift in basic media is defined as: at pH $>$11.0.

TABLE III

CHEMICAL-SHIFT DIFFERENCE OF THE AMINO ACID CARBON ATOMS OF GLYCOSYLATED AND NONGLYCOSYLATED TRIPEPTIDES[a]

Carbon atom	*Δ(Glycosylated tripeptide − nonglycosylated tripeptide)*	
	(1 − 2)	(3 − 4)
Gly C-2 (N-terminal)	−0.1	−0.2
Gly C-2 (internal)	−0.2	
Gly C-2 (C-terminal)		+0.3
Thr C-2	−1.4	−1.2
Thr C-3	+7.6	+7.7
Thr C-4	−0.9	−0.8

[a]The difference in chemical-shift values taken from the ^{13}C-n.m.r. spectra of the compounds at neutral pH.

expected, a downfield shift of 7.6 p.p.m. for C-3. On glycosylation, the signal for C-2 of Thr generally shifts upfield ~1.2 p.p.m., and that of C-4 of Thr shifts upfield ~0.7 p.p.m. It may be noted that there is a slight variance in these shifts when comparing the two different types of compounds (where Thr is either an internal unit, or at the N-terminal position). This variance may be either some kind of inductive effect or, possibly, a slight conformational effect due to glycosylation. This slight difference in the chemical shift is also similarly observed for the glycine residue (internal or N-terminal; see Table III).

Glycopeptide 3 constitutes the first example of a model compound in which the attached carbohydrate group is not directly near a charged species, as the α-D-Gal group is attached to the internal Thr residue. In this case, the carbohydrate carbon resonances exhibit a pH-dependence of ≤ 0.2 p.p.m. (see Table II). Therefore, the chemical-shift data for Thr and α-D-Gal of this compound should be representative of a true model for this type of linkage found in glycoproteins.

In conclusion, we have presented ^{13}C-n.m.r. data for a glycosylated tripeptide that should mimic the true glycosyl linkage pair of Thr and D-Gal found in glycoproteins. Moreover, we give data for glycosylated and nonglycosylated tripeptides that indicate that, in these cases, no large structural perturbations are found as a result of glycosylation.

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