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Note

^{13}C-N.m.r spectral study of 3-*O*-α- and -β-D-xylopyranosyl-L-serine and -threonine

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D-Xylose is one of the few carbohydrates present in the oligosaccharide chains of glycoproteins[1]. It has been found either as a terminal group or as the first residue of the oligosaccharide moiety covalently attached to the peptide backbone of certain glycoproteins[1,2], such as glycosaminoglycans ("mucopolysaccharides") when it is attached to O-3 of L-serine. Elucidation of this carbohydrate–protein linkage has relied on the classical, destructive techniques utilizing chemical modification and, possibly, protein digestion[1].

^{13}C-N.m.r. spectroscopy has recently been employed to gain structural and dynamic information about glycoproteins[3–5], but the use of this technique is currently limited by the nonavailability of ^{13}C-n.m.r. data for model compounds. Therefore, such data for *O*-D-xylosylated L-serine and L-threonine may facilitate the use of this nondestructive technique for the study of glycoproteins containing this sugar, especially glycosaminoglycans. We now present ^{13}C-n.m.r.-spectral data for the biologically relevant, model compounds 3-*O*-α- and -β-D-xylopyranosyl-L-serine (α- and β-D-Xyl*p*→L-Ser) and 3-*O*-α- and -β-D-xylopyranosyl-L-threonine (α- and β-D-Xyl*p*→L-Thr).

EXPERIMENTAL

Materials and methods. — The glycopeptides α- and β-D-Xyl*p*→L-Ser and α- and β-D-Xyl*p*→L-Thr were synthesized as described[6]. Samples for n.m.r. spectroscopy were prepared by dissolving the glycopeptide in de-ionized, distilled water. Adjustments of the pH were made with M NaOH, or HCl, using a Radiometer PHM63 digital pH-meter.

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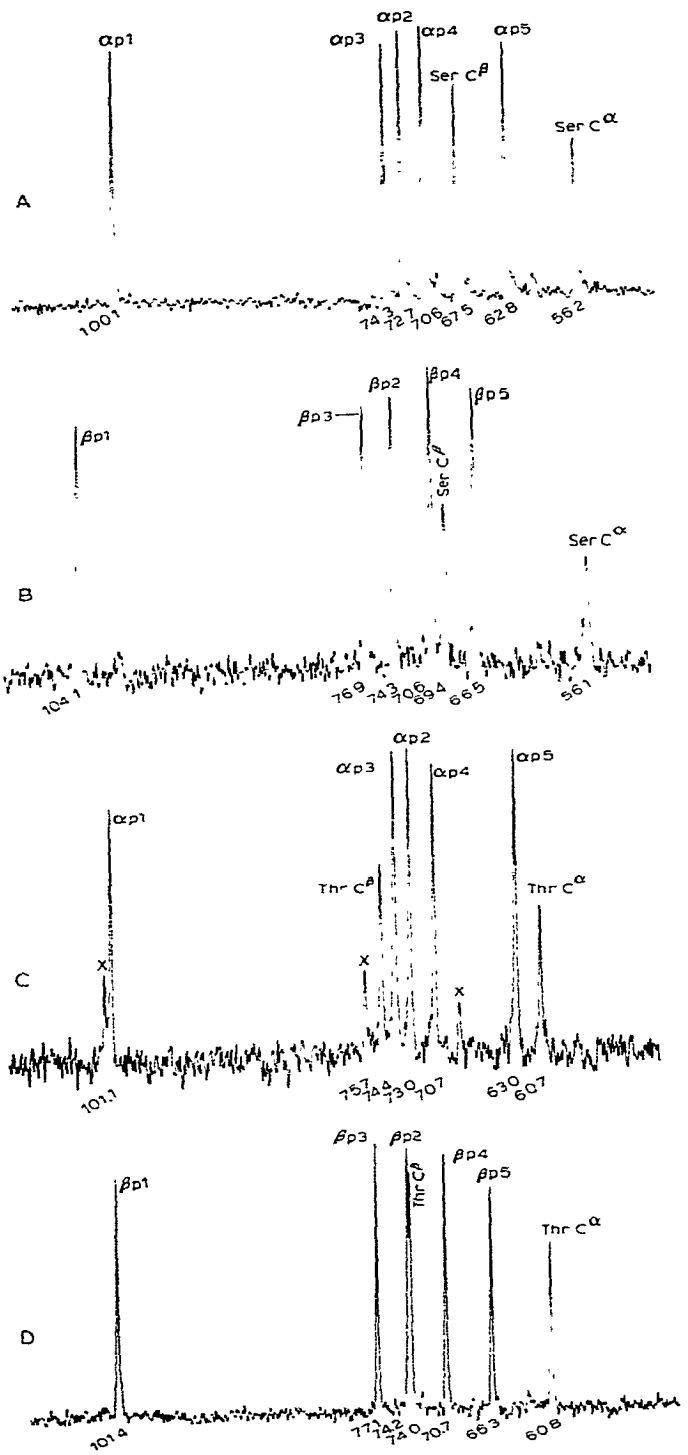

Fig. 1. Aliphatic, carbon-atom region (except C-3 of threonine) of the proton-decoupled, natural-abundance, ^{13}C-n.m.r. spectra of α-D-Xyl*p*→L-Ser, β-D-Xyl*p*→L-Ser, α-D-Xyl*p*→L-Thr, and β-D-Xyl*p*→L-Thr. [Spectra were recorded with a spectral window of 5.500 kHz, and a line broadening of 1.5 Hz was applied during the processing. The notation *αp* refers to the α-pyranose anomer. The number following each *αp* designates the carbon atom of the carbohydrate. The number below each resonance indicates the chemical shift of that resonance. (A) 139mM α-D-Xyl*p*→L-Ser in H_2O, pH 6.7; 9,520 accumulations, using a recycle time of 2 s. (B) 195mM β-D-Xyl*p*→L-Ser in H_2O, pH 7.4; 8,800 accumulations, using a recycle time of 2 s. (C) 150mM α-D-Xyl*p*→L-Thr in H_2O, pH 6.1; 10,000 accumulations, using a recycle time of 2 s. The chemical shift for C-3 of L-Thr was 20.0 p.p.m. (D) 205mM β-D-Xyl*p*→L-Thr, pH 7.3; 12,000 accumulations, using a recycle time of 2 s. The chemical shift for C-3 of L-Thr was 17.8 p.p.m.].

^{13}C-N.m.r. spectra were recorded with a JEOL-FX90Q instrument operating at 21 kG 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° radio-frequency 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. A spectral window of 5.500 kHz was used for recording the spectra. Fully proton-decoupled spectra were obtained when the noise-modulated, ^{1}H irradiation, having a bandwidth of 1.000 kHz, was centered ~4 p.p.m. downfield from Me_4Si. Spectra with ^{13}C–^{1}H coupling were obtained by using a proton-decoupling technique the reverse of that employed for n.O.e. measurements[7].

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

In Fig. 1, A, B, C, and D show the aliphatic region of the proton-decoupled, natural-abundance, ^{13}C-n.m.r. spectra of α-D-Xyl*p*→L-Ser, β-D-Xyl*p*→L-Ser, α-D-Xyl*p*→L-Thr, and β-D-Xyl*p*→L-Thr, respectively (except for C-3 of L-Thr) in aqueous solution. The L-Ser and L-Thr glycosylated with β-D-Xyl*p* were readily differentiated from the α-D-Xyl*p* model compounds on the basis of their anomeric-carbon coupling-constants ($^1J_{CH}$). All β-D-Xyl*p* model compounds exhibited a $^1J_{CH}$ value of 162 Hz, whereas the α-D-Xyl*p* model compounds had an anomeric $^1J_{CH}$ value of 172 Hz, data in agreement with previously published work on coupling constants for α- and β-D-xylopyranose[8,9].

We have placed emphasis on the anomeric coupling-constants for determining the anomeric state of the D-xylosyl group (also chemical shifts of other carbon resonances; see later) instead of relying on the chemical shift of the anomeric carbon atom. From the chemical shifts of methyl α- and β-D-xylopyranoside[10], it would seem that the difference in anomeric chemical-shifts (4.5 p.p.m.) would allow their use in identifying the anomeric state of a D-xylopyranosyl group attached to L-serine or L-threonine in glycoproteins. The data from Fig. 1A (α-D-Xyl*p*→L-Ser) and Fig. 1B (β-D-Xyl*p*→L-Ser) would seem to support this point (difference of anomeric chemical-shifts of 4 p.p.m.). However, the chemical shifts of the anomeric carbon atoms of the α- and β-D-xylopyranosyl group attached to L-threonine are essentially the same; this is due to the *exo*-anomeric effect[11]. This effect makes precarious the use of anomeric chemical-shifts as the sole criteria for determining the anomeric state of D-xylopyranosyl residues attached to the protein backbone.

The assignments of the resonances in the spectra to specific carbon atoms of the carbohydrate were straightforward. They were based on the chemical shifts for methyl α- and β-D-xylopyranoside[10] and for oligosaccharides containing D-xylose[9,12,13]. The assignments for the carbon atoms of the protein were based on chemical shifts

TABLE I

pH-DEPENDENCE[a] OF THE CHEMICAL SHIFTS OF THE PROTEIN CARBON ATOMS, AS DEDUCED FROM THE MODEL COMPOUNDS α-Xyl→Ser, β-Xyl→Ser, α-Xyl→Thr, AND β-Xyl→Thr

Model compound	*C-1′*	*C-2′*	*C-3′*
α-D-Xyl*p*→L-Ser	2.7	5.6	
β-D-Xyl*p*→L-Ser	2.7	6.6	
α-D-Xyl*p*→L-Thr	2.3	3.4	~0.2[b]
β-D-Xyl*p*→L-Thr	3.1	4.5	~0.2[c]

[a]The pH-dependence was determined from the spectra recorded for samples at pH values <2.0 and >11.0. The C-1′ and C-2′ resonances shifted downfield when going from low to high pH. The C-3′ resonances shifted downfield by ~0.2 p.p.m. when going from low to neutral pH, and then shifted upfield by 0.4 p.p.m. when going from neutral to high pH. [b]The chemical shift of C-3′ at neutral pH was 20.0 p.p.m. [c]The chemical shift of C-3′ at neutral pH was 17.8 p.p.m.

reported in the literature[1-4] for peptides containing L-Ser and L-Thr, and also on the pH-dependence of the chemical shifts of the α and β carbon atoms (C-1′ and C-2′; see Table I). Due to glycosylation, the signal for C-2 of L-Ser and L-Thr moves ~5–8 p.p.m. downfield.

Although the α and β carbon atoms exhibited a large pH-dependence, the resonances of the carbohydrate carbon atoms shifted little (all, <0.4 p.p.m.); this suggests that the ^{13}C-n.m.r. data for the D-xylosylated compounds are good models for the carbohydrate residues of glycoproteins, because peptide-bond formation should have little effect on the carbohydrate chemical-shifts.

The data for C-3′ of α- and β-D-Xyl*p*→L-Thr are given in Table I; the signal for C-3′ does not show a significant pH-dependence. This resonance may be a good probe for indicating the anomeric state of a D-xylosyl residue attached to L-threonine, because there is a chemical-shift difference of 2.2 p.p.m. for C-3′ of α- and β-D-Xyl*p*→L-Thr (20.0 p.p.m. for the α anomer, and 17.8 p.p.m. for the β anomer). In theory, C-3 of L-threonine might be a useful probe for determining the anomeric state of a D-xylosyl residue attached to L-threonine in a glycoprotein, but in practice, the region of 19 p.p.m. usually contains many protein resonances (see the spectra in ref. 3).

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