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Measurement of long-range carbon–carbon coupling constants in a uniformly enriched complex polysaccharide

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Abstract

A quantitative coherence transfer scheme for ^{1}H-detected measurement of long-range carbon–carbon coupling constants in NMR spectra of complex carbohydrates is described. It is applied to a uniformly highly ^{13}C-enriched monosaccharide and to a complex cell wall polysaccharide from *Streptococcus mitis* J22 having seven distinct sugars in the repeating subunit. Coupling values within the ring were compared to published values for monosaccharides to demonstrate the validity of the method. An attempt was made to relate coupling constants between carbon atoms across the glycosidic linkage to the dihedral angles of a recently published flexible model for the polysaccharide which is based on $^{3}J_{CH}$ data. The experimental coupling constants do not agree with any single conformation demonstrating that the repeating subunit of the polysaccharide must be flexible. This conclusion is in accord with results of molecular modeling, nuclear Overhauser effect and $^{3}J_{CH}$ data.

Keywords: Polysaccharide conformation; Carbon–carbon coupling; NMR spectroscopy; Oligosaccharide

1. Introduction

Factors influencing the conformation and the dynamics of complex carbohydrate epitopes are not completely understood. Although some workers have proposed that the conformations are relatively rigid and are imposed by stereochemical interactions [1–3], others have emphasized the flexible nature of the oligosaccharide structures [4,5]. Most conformational models of complex oligosaccharides are derived from NOE data in NMR spectroscopy and oligosaccharide models based on a few NOE distance constraints combined with computer molecular modeling have been satisfactory for molecules which are reasonably rigid [6–8]. This treatment has been less successful for more flexible molecules [9].

Scalar coupling data can be a valuable complement to the NOE in oligosaccharides since the J values between atoms across the glycosidic linkage report directly on the glycosidic dihedral angles. J-coupling has additional advantages in averaging over multiple conformations of flexible

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oligosaccharides. For NOE data, averaging over multiple conformations is complicated both by the $1/r^6$ dependence on distance and by the complex and poorly understood time dependence in which the overall molecular tumbling may interact with kinetics of the conformational exchange involved in the internal motion. Averaging of scalar coupling values over multiple conformations is a simple linear average over individual conformers.

The potential value of ^{13}C coupling data between atoms on opposite sides of the glycosidic linkage has long been recognized [10,11], and trigonometric correlation curves relating $^3J_{CH}$ to the glycosidic dihedral angles, Φ and Ψ, have been proposed [12,13]. $^3J_{CH}$ data have been measured for complex oligosaccharides studied in natural abundance ^{13}C using indirect detection [14–17]. With complex polysaccharides enriched in ^{13}C, a large number of relevant coupling constants can be readily measured for high molecular weight polysaccharides with good accuracy, even for small values of $^3J_{CH}$ [18–20].

As a complement to $^3J_{CH}$ data, $^nJ_{CC}$ could provide information useful in resolving the ambiguity resulting from the multi-valued coupling constant correlation curves as well as more data to determine statistical weights of multiple conformations in flexible models. $^nJ_{CC}$ values have been reported in monosaccharides and simple disaccharides [21–23] but no experimental method has been described suitable for high molecular weight polysaccharides or complex oligosaccharides. In the present communication, we describe the application of a method reported by Bax et al. [24] for ^1H-detected measurement of $^3J_{CC}$ of methyl resonances in proteins to complex carbohydrates, including high molecular weight complex polysaccharides.

2. Experimental

Uniformly ^{13}C-enriched D-glucose (99 atom-% ^{13}C) was purchased from Isotec, Inc. The cell wall lectin receptor polysaccharide from *Streptococcus mitis* J22 was extracted from bacteria grown in media containing the uniformly enriched glucose and the product polysaccharide was labeled to an extent of about 96% ^{13}C [18]. This polysaccharide (Fig. 1), having seven sugar residues in the repeating subunit [25], was dissolved in D_2O at a concentration of 8 mg/mL corresponding to an approximate concentration of 8 m*M*. NMR spectra were recorded on a GE Omega 600 spectrometer using a Bruker triple resonance probe at 24 °C. Data were recorded with the pulse sequence of Bax et al. [24]. Short ^{13}C 90° pulse lengths (15 μs in these experiments) are important for this experiment in order to excite coupled carbon atoms with uniform power and phase. The delay, T, was set to correspond to 44 or 46 Hz to minimize the influence of $^1J_{CC}$ and the delay, τ, for transfer of magnetization by $^1J_{CH}$ was set to correspond to 168 Hz. Coupling constants between Ca and Cb were extracted, using eq (1), from the ratio of the cross peak intensity between the proton Ha (attached to Ca) and Cb and the direct peak between Ha and Ca [24].

$$I_{Ha-Cb}/I_{Ha-Ca} = \tan^2(2\pi^n J_{CaCb} T) \qquad (1)$$

The ratio was calculated from a scaling factor which was adjusted for best match between the reference peak and the cross peak.

3. Results and discussion

The pulse sequence used gives a 2-D C–H correlation spectrum in which the magnitude of a cross peak to a ^{13}C resonance is related by eq (1) to the $^nJ_{CC}$ between that carbon atom and the carbon atom attached to the proton in the column. The large peak between the proton and its directly bonded ^{13}C is used as a reference peak in the calculation and the effect of the one-bond ^{13}C–^{13}C coupling to the adjacent carbon atom is minimized by choice of the delay period, T, in the pulse sequence of Bax et al. [24]. Fig. 2 shows ^1H signals from two anomeric proton signals with cross peaks to carbon atoms of the same residue and to the carbon atoms of the succeeding residue of the polysaccharide sequence.

As a control on the precision and accuracy of the $^nJ_{CC}$ measurements, we recorded data for uniformly enriched D-glucose for comparison with values reported previously by Serianni and coworkers [21,22], who measured splittings in direct ^{13}C-detected spectra of monosaccharides enriched with ^{13}C at single sites. For D-glucose (Table 1), our measured values agree within the stated experimental error (± 0.3 Hz).

For the cell wall polysaccharide of *S. mitis* J22 (the individual residues are identified by letters in the structure in Fig. 1), measurements of intraring

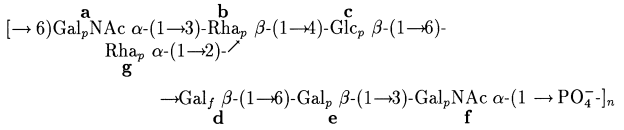

Fig. 1. Structure of the repeating subunit of the cell wall lectin receptor polysaccharide from *Streptococcus mitis* J22

$^3J_{CC}$ are expected to be very similar to values reported for monosaccharides since they depend primarily on puckering of the sugar ring. Data in Table 2 show that the measured values for residue **c** agree with values reported for β-Glc and for residue **e** agree with published data for β-Gal. $^nJ_{CC}$ values measured for residues **f** and **a** (both α-GalNAc) agree well with data reported for α-Gal suggesting only a small effect of the NAc substituent at C2. Measured values for residues **b** and **g** (β-and α-rhamnose, respectively) agree with reported values for β-and α-mannose with the exception of our measured $^2J_{CC}$ for **b** C1–C3 (5.0 Hz) which differs substantially from the corresponding value (4.0 Hz) for β-Man. It is possible that the failure of some of the intraring $^nJ_{CC}$ values measured for the polysaccharide to agree with those for simple monosaccharides could result from effects of substituents in the polymer.

Several types of cross peaks relevant to the conformation of polysaccharides are seen in the spectrum. In Fig. 2 on the column of the anomeric ^{1}H signal, cross peaks are seen representing $^3J_{CC}$ between C1 and Cx ± 1 which are related to the glycosidic dihedral angle, Ψ. Most of these cross peaks can be detected and are reported in Table 2. The technical problems of measuring the relevant coupling constants differ somewhat from those of methyl groups of protein side chains reported by Bax et al. [24] as a result of differences in the chemical shifts. While the most accurate quantitation of this coupling constant can be determined from the column corresponding to the anomeric proton signal, any small residual antiphase magnetization in the spectrum makes it difficult to phase the spectrum correctly if the coupling constant is smaller than about 1.5 Hz. The same coupling constant can also be measured on the columns of Hx ± 1 (data not shown). Unfortunately, as a result of the poor chemical shift dispersion of carbohydrates, this isolated C1-Hx cross peak cannot be easily interpreted if the signal of Hx overlaps with that of another proton whose attached carbon could be coupled to C1. But the cross peak from Hx ± 1 to C1 is in an uncrowded region and can be accurately phased to ascertain whether the $^nJ_{CC}$ value is greater than 1 Hz or is truly zero. The $^nJ_{CC}$ values greater than 1 Hz in Table 1 have a precision

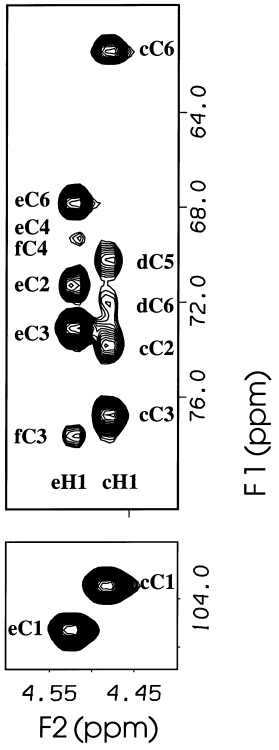

Fig. 2. 2-D quantitative coherence transfer correlation spectrum of the lectin-receptor polysaccharide from *S. mitis* J22. Columns at the ^{1}H resonances of residue **e** β-Gal which is linked (1→3) to residue **f** α-GalNAc. Strong cross peaks correspond to large coupling constants to C3 and C6 of that residue and to C3 of **f**. The resonance of **f** C2 (δ = 49.5 ppm) is too far from **e** C1 for simultaneous 90° excitation and the weak peaks to **f** C4 and **e** C4 overlap. Column at the ^{1}H resonance of residue **c** shows cross peaks corresponding to coupling of **c** C1 (β-glucose) with large coupling constants to C3 and C6 and smaller coupling constants to **d** C6 (linkage position) and to **d** C5 (Cx-1). See ref. [25] for ^{1}H and ^{13}C chemical shift assignments

Table 1
$^nJ_{CC}$ measured for uniformly ^{13}C-enriched D-glucose (in Hz)

Carbon	α-Glc		β-Glc	
	meas.	lit.[a]	meas.	lit.
C1–C3	0	0	4.5	4.6
C1–C4	0	0	0	0
C1–C5	2.1	1.8	ol[b]	0
C1–C6	3.4	3.3	4.0	4.1
C3–C6	ol	3.9	4.5	4.4

[a] Literature values from King-Morris and Serianni [21] and Wu et al. [22].
[b] ol indicates that overlapping cross peaks prevent accurate measurement.

Table 2
$^{n}J_{CC}$(Hz) for the polysaccaride of *S. mitis* J22 (see Fig. 1 for residue identification)

Carbon	Residue													
	a		**b**		**g**		**c**		**d**		**e**		**f**	
	meas.	lit.[a]	meas.	lit.	meas.	lit.	meas.	lit.	meas.	lit.	meas.	lit.	meas.	lit.
C1–C3	0[c]	0	5.05	4.0	0	0	4.25	4.5	3.42	—	4.9	4.6	0	0
C1–C4	0	0	0	0	0	0	0	0	0	—	0	0	0	0
C1–C5	1.64	1.9	0	0	1.53	2.0	0	0	1.79	—	0	0	1.98	1.9
C1–C6	3.48	3.6	lim[b]		lim		3.68	4.1	—	—	4.20	4.4	3.57	3.6
	meas.	calc.[e]	meas.	calc.	meas.	calc.	meas.	calc.	meas.	calc.	meas.	calc.	meas.	calc.
C1–Cx	1.77		1.80		2.28		1.40		1.70		2.04		—	—
C1–Cx−1	0	1.14	0	0.33	ol[d]C1	3.62	2.19	2.85	2.48	4.0	lim		—	—
C1–Cx+1	2.49	3.06	1.94	3.95	0	0.39	—	—	—	—	0	0.38	—	—
C2–Cx	1.73	3.88	2.26	3.13	ol aC5	3.98	0	3.94	2.61	1.64	ol	3.88	—	—

[a] Literature values from data on similar sugars [21,22].
[b] lim. indicates that the ^{13}C chemical shift difference is too large for simultaneous excitation with our instrument.
[c] 0 means that $^{n}J_{cc}$ is less than 1 Hz in our data and less than 0.8 Hz in data of King-Morris and Serianni [21].
[d] ol indicates that overlapping cross peaks prevent accurate measurement.
[e] Calculated from eq (1) with A = 2.24, B = −1.3 and C = 0.5 using the three conformation model given in Tables 5 and 6 of Xu and Bush [19].

of ±0.3 Hz while values lower than 1 Hz are less precise because the peak becomes comparable to noise due to the $\tan^2$ relationship in the trigonometric formula (eq (1)) which relates cross peak intensity to $^{n}J_{CC}$.

While $^{3}J_{C2\text{-}CX}$ values, which are related to the glycosidic dihedral angle Φ, are potentially valuable, their measurement is complicated by overlap of the signals of H2, Hx, C2 and Cx which are often in crowded regions of the spectrum. Four values are reported in Table 1. It has been recently proposed that the two-bond coupling, $^{2}J_{C1\text{-}CX}$, is related to this same dihedral angle [26,27]. The magnitudes, but not the signs, of these coupling constants are reported in Table 1.

The values of $^{3}J_{CC}$ are related to the glycosidic dihedral angles by a trigonometric relation of the form of eq (2).

$$^{3}J_{CC} = A\cos^2\Phi + B\cos\Phi + C \qquad (2)$$

While such a correlation has been proposed for $^{3}J_{CC}$ of the carbonyl carbon atoms of the peptide backbone [28], no explicit correlation has yet been proposed for carbohydrates. Parameterization of the correlation requires coupling data for three known conformations of which two can be extracted from Table 2. While $^{3}J_{C1-C6}$ corresponds to a *trans* conformation, a range of coupling values (3.3 to 4.1 Hz) is observed as a result of the effects of electronegative substituents. We choose 4 Hz which may be an overestimate. $^{3}J_{C1-C4}$ represents a gauche conformation but is complicated by two coupling pathways. Under the assumption that both $^{3}J_{CC}$ pathways make positive contributions to the small measured value of $^{3}J_{C1-C4}$, a small value for the relevant gauche coupling is implied and we choose 0.4 Hz. Selection of C = 0.5 in eq (1) provides a plausible trial correlation function. We recognize that this underdetermined choice of parameters cannot be used for any detailed interpretation and that it does not account for substituent effects. But it does allow for crude estimates of the glycosidic dihedral angles from $^{3}J_{CC}$ in the absence of a better documented correlation.

Table 2, we include values of $^{3}J_{CC}$ calculated from the flexible model for the J22 polysaccharide proposed earlier [19]. There is some disagreement between experiment and values calculated from the model which includes statistical weights of three conformations. Several of the calculated values lie well outside experimental error and the discrepancy is of a fairly fundamental nature. For the glycosidic dihedral angles Ψ_{ab} and Ψ_{bc} between the α-GalNAc and β-Rha residue and between the β-Rha and β-Glc residue, the reported values of $^{3}J_{CH}$ are small (1.5 and 2.1 Hz, respectively) [19], implying that C1 is nearly gauche to the aglycone proton. Thus, for any single conformation, C1 must be nearly *trans* to either Cx+1 or to Cx−1, but in fact the data of Table 2 show both $^{3}J_{CC}$ values to be much less than 4 Hz. Either the coupling constant correlation predicts values of $^{3}J_{CH}$ and $^{3}J_{CC}$ that are too large or the model has too few conforma-

tions. ^{13}C NMR relaxation data on this polysaccharide suggest that the latter defect is the source of the discrepancy [29]. NOE data are not very sensitive to details of the model since simulated NOE from the model of Xu and Bush [19] agree fairly well with the experimental data. Better validation of the $^{3}J_{CH}$ and $^{3}J_{CC}$ coupling constant correlation curves is needed including more data on relatively rigid oligosaccharides such as blood group epitopes for which one can be confident about the model conformation.

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