FEBS Letters 436 (1998) 128–130 FEBS 20873

Residual dipolar couplings as new conformational restraints in isotopically ^{13}C-enriched oligosaccharides

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Received 4 May 1998; revised version received 9 June 1998

Abstract We report the measurement of residual dipolar couplings for ^{13}C-enriched NeuNAcα2-3Galβ1-4Glc in a dilute liquid-crystalline medium. These couplings provide long-range conformational restraints that hitherto have not been available for oligosaccharides. We utilise these restraints in dynamical simulated annealing calculations, which support current models of the solution behaviour of the trisaccharide.

Key words: Residual dipolar coupling; Oligosaccharide; NMR; Gm_3

1. Introduction

The determination of the solution structures of macromolecules by NMR has relied until recently upon ^{1}H-^{1}H nuclear Overhauser effect (NOE) and homo- and heteronuclear long-range spin-coupling constant measurements, giving distance and angular information, respectively. However, these parameters provide conformational information that is inherently short-range in nature. In the case of extended biopolymers such as oligosaccharides, these short-range conformational parameters, when utilised as conformational restraints in dynamical simulated annealing [1] or molecular dynamics simulations [2–7], may not be sufficient to define accurately the solution behaviour. Recently, a series of studies has demonstrated that long-range structural information can be obtained in isotopically enriched proteins by measurement of residual dipolar couplings, either as a result of the small degree of inherent magnetic alignment of the macromolecule [8–11], or by imposing a substantially greater degree of alignment by use of dilute liquid-crystalline solvents [12–14]. Here, we illustrate that the latter approach is suitable for the measurement of residual dipolar couplings in oligosaccharides, with reference to the trisaccharide moiety derived from ganglioside Gm_3 (Gm_3-OS, Neu5Acα2-3Galβ1-4Glc, Fig. 1), which we recently synthesised in ^{13}C-enriched form [15]. Moreover, we demonstrate that these couplings are consistent with a previous model of the solution behaviour of this glycan.

2. Materials and methods

2.1. Sample preparation

NMR samples were prepared by dissolution of 2 mg ^{13}C-enriched (97%) Gm_3-OS prepared as described [15], in a 7.5% solution of dihexanoylphosphatidylcholine (DHPC):dimyristoylphosphatidylcholine (DMPC) (1:2.9, w/w) in D_2O, p*D* 7.2. DHPC and DMPC were purchased from Avanti Polar Lipids (Alabama, USA). The liquid-crystalline phase was obtained by rapid heating from 4°C to 35°C, and in order to stabilise this phase for charged oligosaccharides such as Gm_3-OS, in our hands it is necessary to add KCl to the solution at a concentration of 100 mM.

2.2. NMR experiments

All NMR spectra were recorded on a Varian Unityplus 500 MHz spectrometer. Residual dipolar couplings were obtained as the difference between the one-bond ^{13}C-^{1}H splittings measured at 22°C (isotropic, disordered state) and 35°C (ordered state) using a slightly modified J-modulated HSQC experiment [10]. Each 2-D spectrum consisted of 128 complex t_1 points and 1024 complex t_2 points, with eight scans per point and with spectral widths of 13 000 Hz and 3000 Hz in F_1 and F_2 respectively. Spectra were acquired with delays $2(T-\Delta)$ of between 18 ms and 26 ms inclusive in 1 ms intervals, giving nine spectra in total.

2.3. Structure calculations

Dynamical simulated annealing calculations [16] were performed using ten random geometries of Gm_3-OS as input, and with application of residual dipolar restraints. The geometric content of these restraints was incorporated into the simulated annealing protocol [1] as described [17], using the program XPLOR [18] modified to incorporate dipolar coupling restraints [17]. A uniform value for the force constant $k_{dipolar}$ of 1 kcal Hz^{-2} was utilised throughout the simulated annealing protocol. This is an order of magnitude lower than the value commonly used for structure calculations using dipolar couplings in proteins [11]. However, only very weak restraints are required in oligosaccharides due to the very much lower number of degrees of freedom in these moieties. The torsion angles φ and ψ are defined as H-1–C-1–O-1–C-X (C-1–C-2–O-2–C-X in the case of Neu5Ac) and C-1–O-1–C-X–H-X (C-2–O-2–C-X–H-X in the case of Neu5Ac), where C-X and H-X are aglyconic atoms.

3. Results and discussion

3.1. Measurement of dipolar couplings

In an initial series of experiments (data not shown), suitable conditions were sought for the measurement of residual dipo-

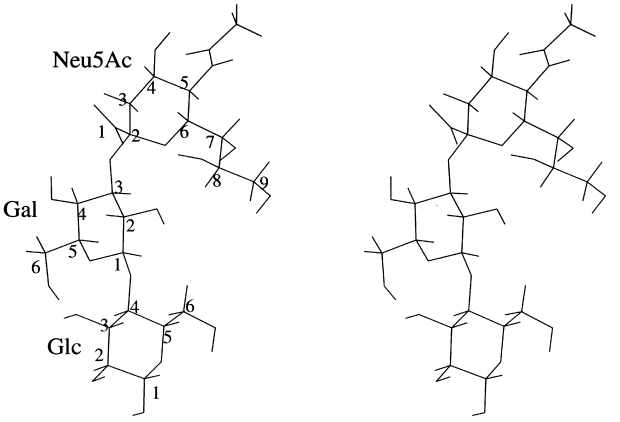

Fig. 1. Stereo view of the trisaccharide Neu5Acα2-3Galβ1-4Glc showing the carbon atom numbering scheme utilised in this study.

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PII: S0014-5793(98)01112-0

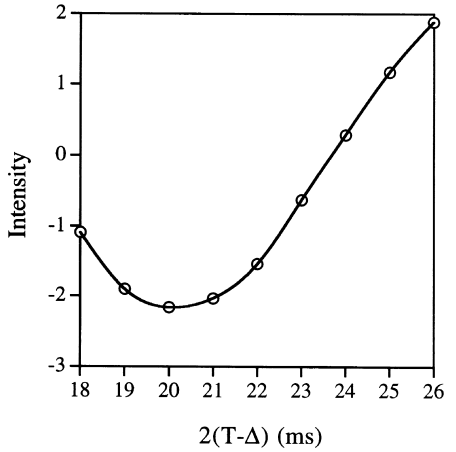

Fig. 2. Typical plot for the peak intensity of the correlation between C-4–H-4 of Galβ as a function of the J-evolution time in the J-modulated HSQC experiment for the measurement of residual dipolar couplings in the trisaccharide. The solid line is a non-linear least-squares fit to the theoretical expression for the J modulation [10].

lar couplings in the trisaccharide, by varying the concentration of DHPC:DMPC. A concentration of 7.5% (w/v) was found to represent a good compromise between the magnitudes of the residual dipolar couplings together with acceptable resonance linewidths. These couplings were measured by use of a J-modulated HSQC-type experiment (see Section 2). A typical curve obtained by fitting the peak intensity of the C-4–H-4 correlation of Galβ as a function of the J-evolution time is shown in Fig. 2. The resulting dipolar couplings for all non-overlapping resonances are given in Table 1. As anticipated, since the ring geometries of the constituent monosaccharide residues in the oligosaccharide are essentially fixed on the NMR timescale, the relative magnitudes of the intra-residue residual dipolar couplings reflect the stereochemistry at each carbon centre. For example, the residual dipolar couplings for the C-1–H-1, C-2–H-2, C-3–H-3 and C-5–H-5 bond vectors in Galβ are comparable, reflecting a similar (axial) orientation of the methine protons. In contrast, the residual dipolar coupling for the C-4–H-4 bond vector of Galβ is very small, reflecting a different (equatorial) orientation of the methine proton.

Table 1
Residual ^{1}H-^{13}C dipolar couplings for Neu5Acα2-3Galβ1-4Glc in a 7.5% (w/v) solution of DHPC:DMPC (1:2.9 w/w) in D_2O, p*D* 7.2, containing 100 mM KCl

Bond vector	Residual dipolar coupling (Hz)[a]
Glcβ H-1–C-1	+7.4
Glcβ H-2–C-2	+9.5
Glcβ H-3–C-3	+9.6
Glcβ H-4–C-4	+7.7
Galβ H-1–C-1	+11.5
Galβ H-2–C-2	+11.9
Galβ H-3–C-3	+9.2
Galβ H-4–C-4	−1.4
Galβ H-5–C-5	+9.5
Neu5Acα H-4–C-4	+3.2
Neu5Acα H-5–C-5	+4.8
Neu5Acα H-7–C-7	−13.4
Neu5Acα H-8–C-8	−11.1

[a]Values obtained by non-linear least-squares fitting of experimental intensities from J-modulated HSQC experiments [10]. Estimated average error in the measurements is ±0.5 Hz.

3.2. Derivation of components of the alignment tensor

As a first approximation the coupling for the C-7–H-7 bond vector, as the minimum value, can be taken to be aligned perpendicular to the principal axis of the alignment tensor [17], giving rise to a value for the axial component of the alignment tensor $S{\cdot}A_a = 5.75\times10^{-4}$. Using this value and assuming a uniform value for the general order parameter *S*, the rhombic component of the alignment tensor was determined from a series of trial dynamical simulated annealing calculations incorporating dipolar restraints for Galβ only. Since the relative geometries of the endocyclic bond vectors of this residue (or indeed any other) are to first order independent of the overall conformation of the molecule, the rhombic component of the alignment tensor could thus be obtained by simplex optimisation of this parameter with respect to the best fit between experimental and theoretical residual dipolar couplings for Galβ. The best fit was obtained with a rhombicity of 0.2, i.e. $S{\cdot}A_r = 1.15\times10^{-4}$.

3.3. Dynamical simulated annealing calculations

In order to examine the conformational properties of the trisaccharide that give rise to the residual dipolar couplings given in Table 1, the latter were incorporated in a restrained dynamical simulated annealing protocol. This simulation gave rise to a series of low energy structures (Fig. 3), each member of which possessed a single family of conformations about the Neu5Acα2-3Gal glycosidic linkage, with φ, ψ ∼ −72°, +5°, and two families about the Galβ1-4Glc glycosidic linkage, with φ, ψ ∼ +15°, −18° and +62°, +15°, respectively. There were no deviations from experimental dipolar couplings greater than 0.1 Hz, indicating that each conformation is essentially consistent with all of the measured dipolar couplings. In the context of our previous study on the structure and dynamics of Gm_3-OS [7], the conformations about the glycosidic linkages implied from the dipolar restraints map to accessible regions of conformational space defined in that study (Fig. 4), i.e. the measured dipolar restraints are consistent with a model involving relatively small torsional oscillations about the minimum energy conformation of each glycosidic linkage. At first sight it might appear surprising that the residual dipolar couplings alone restrain the molecule as well as is shown in Fig. 1. However, since the internal geometry of each monosaccharide ring is essentially fixed on the NMR timescale, internal motion is restricted to torsional oscillations about the glycosidic linkages, and hence the 'long-range' nature of

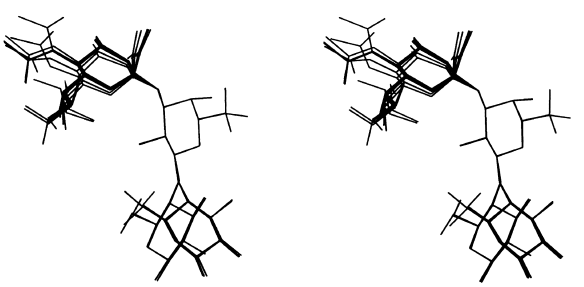

Fig. 3. Stereo view of the lowest energy families of structures derived from dynamical simulated annealing calculations on Neu5Acα2-3Galβ1-4Glc including dipolar restraints given in Table 1.

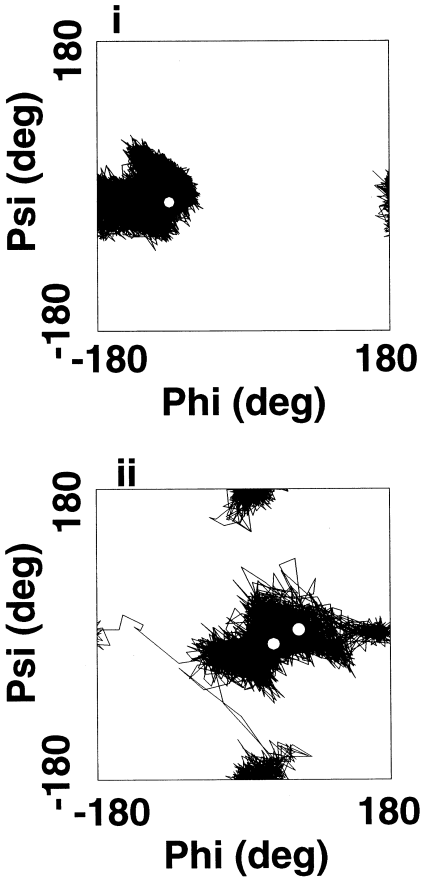

Fig. 4. Plots of the instantaneous values of φ, ψ for (i) Neu5Acα2-3Gal and (ii) Galβ1-4Glc glycosidic linkages of Neu5Acα2-3Galβ1-4Glc derived from the 5 ns molecular dynamics simulation described [7]. The low energy values of φ, ψ predicted from dynamical simulated annealing calculations (Fig. 3) are indicated (○).

the dipolar couplings act as very effective restraints about these linkages in comparison with conventional NOE restraints [7].

The orientation of the *z* axis of the external axis system used in the simulated annealing calculations, which indicates the direction of the principal axis of the alignment tensor, is approximately collinear (within 20°) of the C-5–C-6 bond vector of Neu5Acα. The large negative couplings for the C-7–H-7 and C-8–H-8 bond vectors of the sidechain of Neu5Acα indicate that these vectors are fixed in an orientation perpendicular to the C-5–C-6 bond, consistent with our earlier observations [7], and supporting the hypothesis [19] that this sidechain is stabilised by a hydrogen bond in solution from OH-8 to the carboxyl group of Neu5Ac.

4. Conclusions

We have demonstrated that small oligosaccharides such as Neu5Acα2-3Galβ1-4Glc exhibit sufficient anisotropy in solution to enable a degree of orientation in dilute liquid-crystalline solvents to be obtained sufficient for the measurement of substantial residual dipolar couplings from C-H bond vectors. These dipolar couplings, which contain long-range structural information, suggest a model for the solution behaviour of the trisaccharide that is consistent with a previous study [7] which utilised short-range structural information from ^{1}H-^{1}H nuclear Overhauser effect and three-bond transglycosidic ^{1}H-^{1}H and ^{13}C-^{1}H coupling constant measurements.

Acknowledgements: G.R.K. acknowledges Unilever PLC for the provision of a CASE award.

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