Carbohydrate Research, 198 (1990) 375–380
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Conformational analysis of a disaccharide fragment of the polysaccharide antigen of *Streptococcus pneumoniae* type 1 using n.m.r. spectroscopy and HSEA calculations

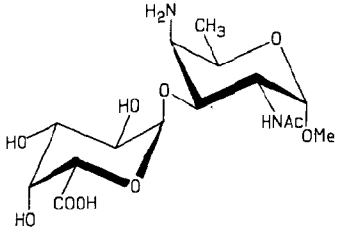
KLAUS BOCK,
Department of Chemistry, Carlsberg Laboratory, Gl. Carlsberg Vej 10. DK-2500 Valby (Denmark)

HANS LÖNN,
Biocarb AB, S-223 70 Lund (Sweden)

AND THOMAS PETERS
Institut für Biophysicalische Chemie, Johann Wolfgang Goethe Universität, Theodor-Stern-Kai 7, D-6000 Frankfurt (F.R.G.)

(Received February 22nd, 1989; accepted for publication, October 4th, 1989)

The capsular polysaccharide of *Streptococcus pneumoniae* type 1 is composed of trisaccharide repeating-units →3)-α-D-Gal*p*A-(1→3)-α-Sug*p*-(1→4)-α-D-Gal*p*A-(1→, in which Sug denotes 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose[1], and recently a disaccharide glycoside [α-D-Gal*p*A-(1→3)-α-Sug*p*-OMe (**1**)] related to this structure has been synthesized[2]. We now report on the conformational analysis of **1**, in order to investigate the conformational preferences of such a charged molecule using the simple approach by n.m.r. spectroscopy and HSEA calculations[3,4].



1

A ^{1}H-n.m.r. spectrum for a solution of **1** in D_2O is shown in Fig. 1A, and the parameters are given in Table I. The assignments, based on a first-order analysis from 1D and 2D spectra, were straightforward. The ^{13}C-n.m.r. data are given also in Table I, and the assignments were based on comparison with data for model compounds[5].

0008-6215/90/$ 03.50

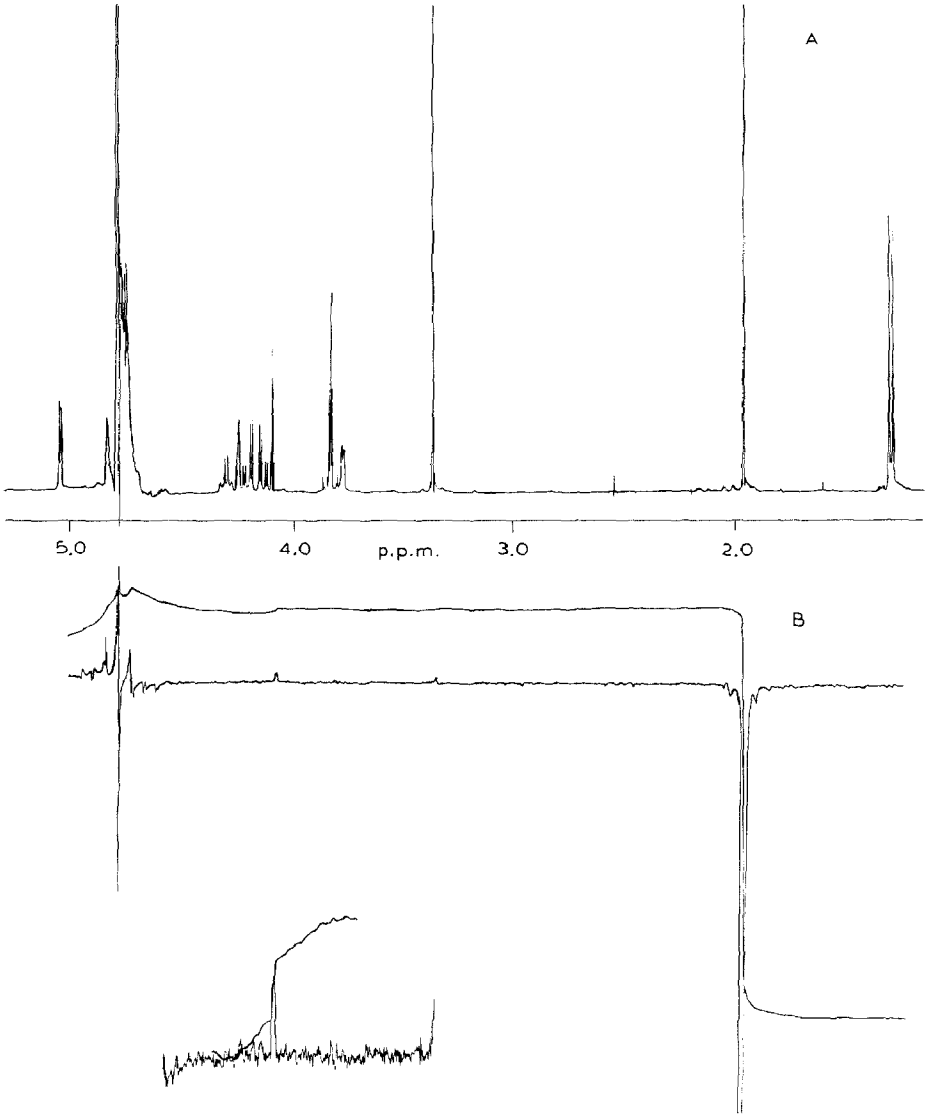


Fig. 1. A, ^{1}H-N.m.r. (500 MHz) spectrum of a solution of **1** in D_2O at 27°; B, difference n.O.e. experiment of **1** showing the result of saturation of the NAc group at 2.00 p.p.m. and the observed enhancement (2.3%) of H-5′ at 4.12 p.p.m.

The conformational preference of **1** was assessed by determination of the n.O.e. on saturation of H-1′, and enhancements of 5.5, 5.0, and 9.2%, respectively, were observed for the resonances of H-3, H-4, and H-2′. Furthermore, saturation of the NAc methyl group caused an enhancement of 2.3% of the resonance of H-5′ (Fig. 1B).

Calculations of the interactions of the two units, using hard-sphere exo-

TABLE I

^{1}H-N.M.R. DATA FOR **1**

^{1}H- (δ in p.p.m., J in Hz)

H-1'	*H-2'*	*H-3'*	*H-4'*	*H-5'*			
5.07	3.88	3.86	4.27	4.12			
3.3	10.2	2.9	1.6				
H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*NAc*	*OMe*
4.78	4.16	4.23	3.82	4.33	1.33	2.00	3.40
3.6	11.2	4.2	1.6	6.6			

^{13}C (δ in p.p.m.)

C-1'	*C-2'*	C-3'	*C-4'*	*C-5'*	*C-6'*		
98.2	68.5	70.6	71.7	73.6	176.4		
C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*NAc*	*OMe*
99.2	48.8	73.6	53.7	63.6	16.7	175.6 23.0	56.6

N.O.e

Proton saturated			*Proton observed*			*Proton saturated*	*Observed*
Observed n.O.e.							
H-1'			H-3	H-4	H-2'	NAc	H-5'
			5.5%	5.0%	9.2%		2.3%
Calculated n.O.e.							
First-order global minimum			3.1%	4.6%	10.1%		3.1%
Full-matrix global minimum							
$\tau_c \times 10^{-9}$	$\tau_i \times 10^{-10}$						
0.26	0.26		2.9%	4.5%	10.4%		3.0%
Full-matrix ensemble average							
$\tau_c \times 10^{-9}$	$\tau_i \times 10^{-10}$	Solvent *Relxation*					
0.1	0.1		7.2	13.9	29.7		5.6
0.4	0.4		−1.4	−2.1	−4.4		0.1
0.29	0.29		2.0	3.3	7.0		1.9
0.26	0.26		2.9	4.9	10.4		2.4
0.23	0.23		3.8	6.5	13.9		3.0
0.26	*0.26*	*5%*	*2.8*	*4.8*	*9.4*		*2.3*
0.26	0.26	10%	2.7	4.6	8.5		2.2
0.26	0.26	25%	2.4	4.2	6.8		1.9

anomeric effect (HSEA) calculations[3,4], gave the ϕ/ψ isoenergy contour map shown in Fig. 2A, with a minimum energy conformation of ϕ_H (H-1'–C-1'–O-1'–C-3)/ψ_H (C-1'–O-1'–C-3–H-3) (−42°/−32°). The orientation of the NAc group was set as found in X-ray structures with the C-2–H-2 and the C=O bonds eclipsed. HSEA calculations do not include hydrogen atoms (such as OH, NH_2, or COOH), which are in rapid exchange, but it proved to be important to include the coordinate

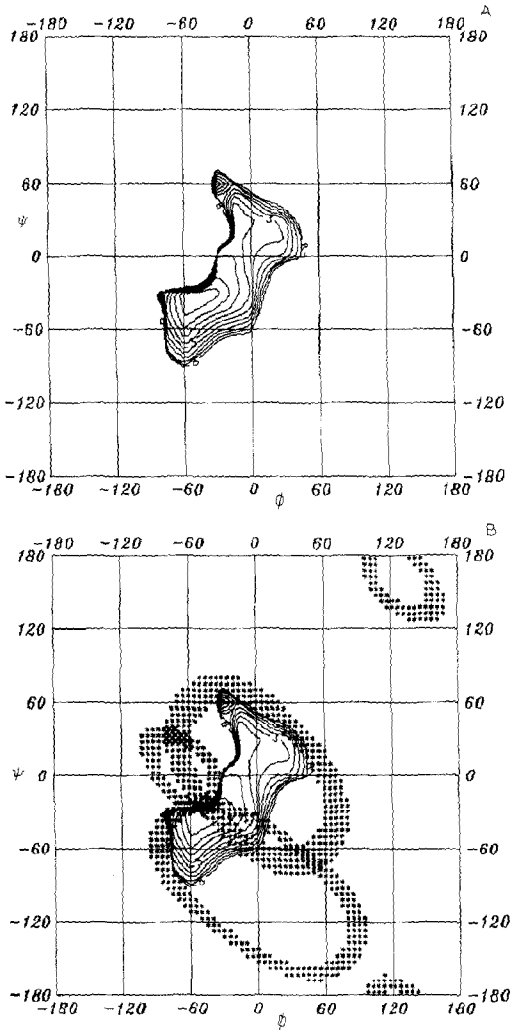


Fig. 2. A, Isoenergy contour diagram for rotation of ϕ_H(−180° to 180°) and ψ_H (−180° to 180°) for compound **1**; B, as in A but overlaid with the experimentally observed distance constraints from H-1′ to H-3 (2.45Å+/−0.15), H-1′ to H-4 (2.45Å+/−0.15), and H-5′ to NAc (2.85Å+/−0.25).

for the NH proton of the NHAc group in the calculations, in order to obtain a good agreement between the experimental data and the calculated conformational preference.

As discussed earlier[4,6], the observation of one inter-residue n.O.e. and its assignment to a distance does not unequivocally determine ϕ_H/ψ_H, but rather imposes restraints on the conformational map. This is demonstrated in Fig. 2B, where the experimentally observed n.O.e.s are presented as distance constraints from H-

1′ to H-3 (2.45Å+/−0.15), H-1′ to H-4 (2.45Å+/−0.15), and H-5′ to NAc (2.85Å+/−0.25). Only conformers with ϕ_H −40° to −60° and ψ_H −20° to −35° will fulfill these experimental observations.

However, a more realistic approach to the evaluation of the experimental data is to use the conformational model to calculate the observed n.O.e.s. In Table I are shown the calculated n.O.e.s from the minimum energy conformation described above, using a simpler r^{-6} dependence[4] or using a full-matrix calculation[7] assuming dipole–dipole relaxation only and isotropic reorientation with correlation times of 0.1–0.4 × 10^{-9}s and 0.1–0.4 × 10^{-10}s for the internal motion of the Me-5 and NAc, respectively, in a three-fold jump model[8]. Furthermore, relaxation contributions from the solvent of 5, 10, and 25% are included in order to evaluate this contribution on the relaxation behaviour. The n.O.e.s are critically dependent on the isotropic correlation time. The agreement is fair, but a better evaluation of the experimental data is expected[9] if the average n.O.e. values are calculated using the whole energy surface (Fig. 2A). Therefore, an ensemble average relaxation matrix was calculated for all conformers in a 10° grid for ϕ and ψ, starting from the minimum calculated above, and then averaging according to a Boltzmann distribution function at 27° for the respective relative energies. These results (Table I) show that the agreement with the experimental data is better than using a single conformer model, and that the best fit is obtained if a solvent relaxation of 5% is included in the calculations and assuming an isotropic reorientation of 0.26 × 10^{-9}s. Thus, it can be demonstrated that the conformational preference of **1** experimentally determined accords with that calculated, even though the charged atoms were treated as neutral spheres with van der Waals radii similar to the neutral atoms.

EXPERIMENTAL

N.m.r. spectra were recorded with a Bruker AM 500 spectrometer operating at 500 MHz for ^{1}H on a 0.1M solution of **1** in D_2O at neutral pH at 27° (internal acetone, 2.22 p.p.m.; DOH at 4.75 p.p.m.). A sweep width of 5000 Hz using 32k of computer memory, giving a digital resolution of 0.3 Hz/point, was used together with pulse angles of 10 μs (60°). COSY experiments were made using Bruker standard software, and the n.O.e. experiments were performed in the difference mode. The ^{13}C-n.m.r. spectra were recorded at 125.77 MHz at 27° (internal 1,4-dioxane, 67.4 p.p.m.). A sweep width of 25 000 Hz using a computer memory of 64k, giving a digital resolution of 0.8 Hz/point, was used together with a pulse angle of 5 μs (90° = 8.5 μs).

The HSEA calculations were performed[4] on a IBM PS/2 system model 80 with a 387 math-coprocessor. The calculations of the ensemble average n.O.e.s were performed[9] on a TITAN (Ardent Computer Systems) computer. The coordinates for the α-D-galacturonic acid residue were taken from the X-ray structure[10] and the protons attached as described[3]. The coordinates for the Sug unit were constructed by bond modification of the X-ray data of 2-acetamido-2-deoxy-α-D-galactose[11], using the program Alchemy[12].

REFERENCES

1 B. LINDBERG, B. LINDQUIST, J. LÖNNGREN, AND D. A. POWELL, *Carbohydr. Res.*, 78 (1980) 111–117.
2 H. LÖNN AND J. LÖNNGREN, *Carbohydr. Res.*, 132 (1984) 39–44.
3 K. BOCK, *Pure Appl. Chem.*, 55 (1983) 605–622.
4 H. THØGERSEN, R. U. LEMIEUX, K. BOCK, AND B. MEYER, *Can. J. Chem.*, 60 (1981) 44–57.
5 K. BOCK AND C. PEDERSEN, *Adv. Carbohydr. Chem. Biochem.*, 41 (1982) 27–66.
6 K. BOCK, T. FREJD, J. KIHLBERG, AND G. MAGNUSSON, *Carbohydr. Res.*, 176 (1988) 253–270.
7 J. H. NOGGLE AND R. E. SCHIRMER, *The Nuclear Overhauser Effect*, Academic Press, New York, 1971.
8 F. HEATLEY, L. AKHTER, AND R. T. BROWN, *J. Chem. Soc., Perkin Trans. 2*, (1980) 919–924.
9 D. A. CUMMING AND J. P. CARVER, *Biochemistry*, 26 (1987) 6664–6676; T. PETERS, D. R. BUNDLE, AND J. R. BRISSON, *Can. J. Chem.*, (1989) submitted for publication.
10 S. THANOMKUL, J. A. HJORTÅS, AND H. SØRUM, *Acta Crystallogr., Sect. B*, 32 (1976) 920–922.
11 P. A. NEUMAN, H. GILLIER-PANDRAUD, AND F. LONGCHAMBON, *Acta Crystallogr., Sect. B*, 31 (1975) 474–477.
12 Alchemy Molecular Modeling Program. Tripos, St. Louis, 1988.