Correlation between the Chemical Shift Values and Precise Interglycosidic Distance Measurements in the Cyclic Glucan of *Burkholderia solanacearum*

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The structure of cyclic osmoregulated periplasmic glucans (OPGs) synthetized by different Gram-negative bacteria might shed light on the role that these molecules play in the adaptation to osmotic stress and in the phytopathogenicity of these bacteria.[1] The cyclic nature of these molecules could be essential for both aspects, but a precise structural model is needed to assess this point. Recently, the structures of two cyclic glucans extracted from cells of *Xanthomonas campestris*[2,3] and *Burkholderia solanacearum*[2] were described. Whereas these molecules share the cyclic nature with OPGs of members of the *Rhizobiaceae* family, they are fundamentally different by the unique degree of polymerization (16 versus 13 units) in contrast to the size distribution found in *Rhizobiaceae* and by the single α-(1−6) linkage in contrast to the all β-(1−2) character of the OPGs found in the *Rhizobiaceae* members. The latter single α-(1−6) linkage makes the ring closure feasible and induces structural constraints in this molecule to such extent that all individual anomeric proton resonances can be distinguished (Figure 1). Its minimal size and the complete resonance assignment of the cyclic OPG from *B. solanacearum* make this molecule an ideal candidate for a detailed structural study.

The chemical shifts of the H1 and C2 atoms flanking the glycosidic β-(1−2) linkage clearly show an alternating pattern[2,3] (Figure 2). On the basis of previous calculations correlating the chemical shift values to conformation,[4] this suggests a nonuniform location of the interglycosidic linkages in the (Φ, Ψ) map.[5] We present here the precise measurement of the interglycosidic distances to clarify the conformational characteristics underlying this alternating pattern. A limited number of studies[6] have attempted to examine the conformational behavior of glycosidic linkages on the basis of precise NOE curves. However, whereas the small number of distance restraints between the two pyranosyl units requires a precise determination of the latter, the flexibility of the glycosidic linkage may lead to wrong distances due to a difference in dynamics with the proton pair considered as the internal distance reference. To take into account the possible variations of internal flexibility, at least two independent measures should be obtained for each pair of protons. The off-resonance ROESY technique[7] allows the measurement of the dipolar cross-relaxation rates along an effective field axis in the rotating frame tilted by an angle θ with respect to the static magnetic field. By variation of this angle from zero to higher values, the longitudinal (σ) and transverse (μ) dipolar cross-relaxation rates can be determined with a high accuracy since they result from an overdetermined fitting procedure. Finally, these two values can be exploited simultaneously to obtain distances without the requirement of any internal reference.[8]

The complexity of the spectrum (Figure 1) impeded the use of selective pulses to record 1D buildup curves. As severe overlaps between the H2 and H5 protons occurred for several residues, a sample enriched in ^{13}C was prepared by replacing

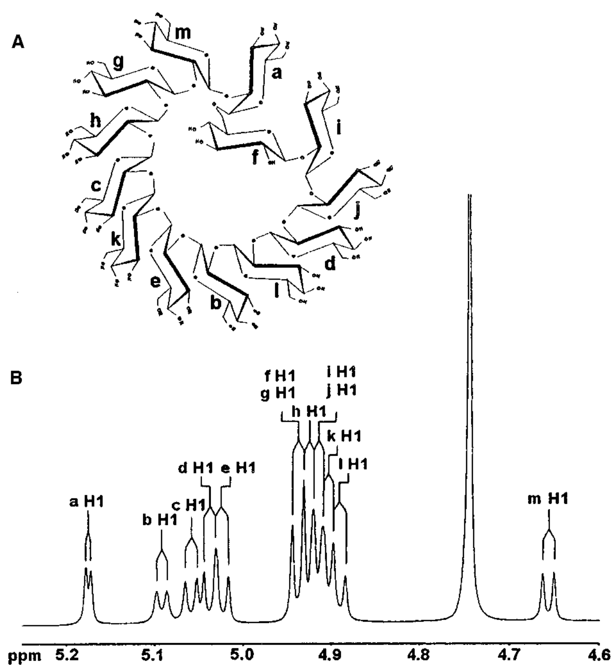

Figure 1. Primary structure (A) and the anomeric proton spectrum (B) of the cyclic OPG of *Burkholderia solanacearum*.

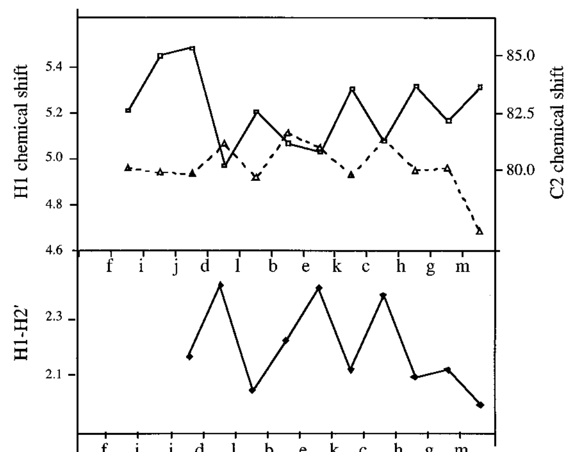

Figure 2. Variations of the H1 (−△−) and C2 (−□−) chemical shift values (upper, ppm) and of the interglycosidic distances H1−H2′ (lower, Å) along the primary sequence.

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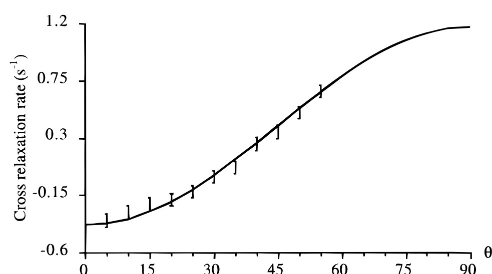

Figure 3. Buildup rates for the contact H1(h)−H2′(g) as a function of the off-resonance angle θ. The solid line is the best fit to $\sigma \cos2(\theta) + \mu \sin2(\theta)$. Cross-peak extraction, buildup curves, and least-squares fitting routines were all programmed within the NMR package SNARF (F. van Hoesel, University of Groningen, The Netherlands).

Table 1. Longitudinal Cross-Relaxation Rates as Determined from the HSQC-NOE Buildup Curves (Column 2) and the HSQC-(Off-Resonance ROESY) Fitting Procedure (Column 3), Transverse Cross-Relaxation Rates by the Same Procedure (Column 4), and Resulting Pairwise Distances r and Dipolar Correlation Times τ_{cp} (Columns 5 and 6)

	HSQC-NOE	HSQC-(off-resonance ROESY)			
	σ (s^{-1})	σ (s^{-1})	μ (s^{-1})	r (Å)	τ_{cp} (ns)
f1−i2	ND[a]	ND	ND	ND	ND
i1−j2	ND	ND	ND	ND	ND
j1−d2	−0.35	−0.37	1.0	2.19	0.9
d1−l2	−0.20	−0.20	0.52	2.47	0.9
l1−b2	−0.40	−0.40	1.43	2.06	0.7
b1−e2	−0.38	−0.38	0.93	2.25	1.0
e1−k2	−0.19	−0.18	0.49	2.46	0.8
k1−c2	−0.33	−0.32	1.10	2.14	0.7
c1−h2	−0.21	−0.20	0.58	2.43	0.8
h1−g2	−0.42	−0.37	1.15	2.09	0.7
g1−m2	−0.39	−0.46	1.22	2.14	0.9
m1−a2	−0.48	−0.59	1.70	2.00	0.8

[a] ND = not determined due to overlap.

one-fourth of the casein hydrolysate used for the nonenriched sample[2] by an equal amount of a mixture of uniformly ^{13}C-enriched amino acids and glucose (≪sugar mix≫, EMBL, Heidelberg, Germany). All measurements were performed on this sample as heteronuclear 1H/13C correlated HSQC-NOESY and HSQC-(off-resonance ROESY) spectra, exploiting the large chemical shift dispersion of the H1 proton and C2 carbon resonances.[2]

Buildup rates for the normalized cross peaks in HSQC-NOESY spectra were determined on the basis of four mixing times between 100 and 400 ms with a good linear behavior up to at least 300 ms. The off-resonance ROESY spectra were recorded as a series of 2D spectra with a constant spin-lock field strength $\gamma B1 = 7$ kHz but with varying offset Δ of the spin-lock field. Eleven values of $\theta = \arctan(\gamma B1/\Delta)$ were sampled between 5° and 55°, with two mixing times of 150 and 300 ms for every angle. Buildup curves were determined independently for every angle, using the cross-peak volumes normalized to the diagonal peak ones in order to extend the validity of the initial slope approximation.[9] A least-squares fit of the resulting slopes as a function of the angle θ to the theoretical expression $\sigma' = \sigma \cos^2(\theta) + \mu \sin^2(\theta)$ yields both relaxation rates (Figure 3). We observe excellent agreement between the σ rates determined by the classical NOE buildup rates and those obtained by the off-resonance ROESY fit (Table 1).[10] The independent determination of σ and μ allows extraction of pairwise structural (r) and dynamic (τ_{cp}) parameters (Table 1, columns 5 and 6).[11] Two classes of interglycosidic distances can be distinguished, those linking a H1 proton from group 1 [b−e] to the H2 proton from group 2 [f−l], with values around 2.45 Å, and those where a unit from group 2 substitutes for a unit from group 1, with significantly[12] shorter distances (around 2.15 Å). An averaged distance between these extremes is found for the pair b1−e2. However, the anomeric proton of residue b is appreciably broadened (around 0.5 Hz at 301 K) compared to the other anomeric resonances (Figure 1). At lower temperatures (288 K), the splitting due to the homonuclear coupling constant disappeared completely due to the line broadening, proving that the glycosidic linkage exhibits some low-frequency jumps between two (or more) positions. The longer correlation time observed for this NOE contact indicates that, in the nanosecond range, this glycosidic bond is more rigidly fixed than are the other glucose units. The b−e glycosidic bond is diametrically opposed to the α-(1−6) linkage between units a and f (Figure 1), and it links two units within group 1; these two observations might be the basis of its particular behavior.

Relaxed energy maps for β-sophorose[13] show the main low-energy region at $20° < \Phi < 80°$ and $-60 < \Psi < 60°$. A comparison of the calculated energy map with a map showing the H1−H2′ interproton distance of β-sophorose as a function of the interglycosidic angles together with the observed strong NOEs indicates that all β-(1−2) bonds of the cyclic OPG of *B. solanacearum* are situated in this main low-energy zone.[2,3] However, the calculated precision of the interglycosidic distances (Table 1) allows us to discern a pattern of alternating shorter and longer H1−H2′ distances along the primary sequence, following closely the alternating pattern of chemical shift values (Figure 2). The deviating distance found for the b1−e2 contact is accompanied by a similar perturbation for the H1 and C2 chemical shift values of this particular linkage (Figure 2).

Application of the off-resonance ROESY technique has allowed a precise measurement of the interglycosidic distances in the cyclic OPG of *B. solanacearum*. All β-(1−2) linkages are located in the zone of lowest energy as calculated for β-sophorose. The alternating pattern observed for the interglycosidic distances correlates very well with the observed pattern of chemical shift values and provides a geometrical basis for the chemical shift parameter. The results presented here form a first step toward a detailed experimental model of the cyclic glucans that might shed light on the biological role of these molecules and allow us to establish whether the cavity of these molecules is compatible with the complexation of host molecular signals.

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(12) Estimating a maximal error of 10% in the cross and diagonal peak volumes, intrinsic upper limits on the error bars are ±0.05 Å for the distances and ±0.04 ns for the correlation times. Of course, these values do not take into account the bias associated with the assumed model of motions. However, it has been demonstrated for an equivalent molecule (ref 11) that the effect of this bias on found distances is smaller than that obtained using an internal distance reference. The distance ranges observed for group 1 and group 2 are therefore significantly different.

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