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CONFORMATION OF (1→6)-β-D-GLUCAN BY VACUUM-ULTRAVIOLET CIRCULAR DICHROISM

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ABSTRACT

Gelation of pustulan [(1→6)-β-D-glucan] likely involves some chain ordering. To characterize the most probable conformation, we previously carried out energy calculations and identified the local energy minimums. Here, we analyze the (previously measured) vacuum-ultraviolet circular dichroism of pustulan by using a quadrant rule recently proposed for the 175-nm c.d. band and our earlier quantum-mechanical model for the 185-nm c.d. band. Most of the local-energy minimum conformations are shown to be inconsistent with the circular dichroism observed. Of the two distinct conformations consistent with the observed circular dichroism, one includes the global-energy minimum calculated earlier, i.e., $(\phi, \psi, \omega) = (20°, -90°, gt)$.

INTRODUCTION

The lowest-energy electronic excitation in unsubstituted polysaccharides occurs at wavelengths shorter than 190 nm, so that, above that wavelength, they display no circular dichroism (c.d.), and their optical rotatory dispersion is featureless, lacking Cotton effects. Measurements below 190 nm are difficult, but, in the past decade, a substantial amount of c.d. data in the vacuum-ultraviolet region (v.u.v. c.d.) has been collected for polysaccharides[1]. We and our collaborators recently proposed[2] an empirical, quadrant rule for the major c.d. band, which occurs in solution near 175 nm. Furthermore, we have developed a semi-empirical, quantum-mechanical model for the weak 185-nm c.d. band that is sometimes observed, apparently only when rotation about the inter-residue linkages is particularly restricted[3].

With these new interpretive tools, we are able to combine v.u.v. c.d. measurements with conformational-energy calculations, in order to draw conclusions about the most probable conformation in solution. Conformational-energy calculations sometimes result in a relatively large number of favored conformations having similar calculated energies, but not all of these putative conformations will be consistent with the observed v.u.v. c.d., as analyzed with the recently developed interpretive models. It might be expected that, in favorable

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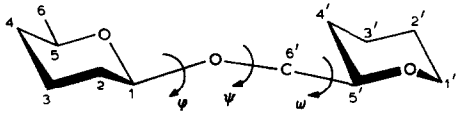

Fig 1 Gentiobiose, a dimer of β-D-glucose, representing the disaccharide repeating unit of the polymer pustulan [(1→6)-β-D-glucan]. $\phi = 0°$ corresponds to C-1–H-1 *cis* to O-1–C-6′, positive values of ϕ represent clockwise rotation of the second residue about the C-1–O-1 bond as viewed from C-1 toward O-1 $\psi = 0°$ corresponds to O-1–C-1 *cis* to C-6′–C-5′, positive values of ψ represent clockwise rotation of the second residue about the O-1′–C-6′ bond as viewed from O-1 to C-6′ Consideration of rotation about the C-6′–C-5′ bond, ω, is limited to the *gg* orientation (C-6′–O-1 *trans to* C-5′–H-5′) and the *gt* orientation (C-6′–O-1 *trans* to C-5′–C-4′)

cases, the c.d. data can be used to screen the low-energy conformations, to yield a very small number of most probable conformations, or even a single conformation.

Here, we apply the method to a conformational analysis of pustulan [(1→6)-β-D-glucan] (see Fig. 1). We initially consider, for the corresponding disaccharide, gentiobiose, all of the energetically favored conformations, which, by virtue of the (1→6) linkage, are rather large in number[4,5]. We next apply our recently proposed quadrant rule[2] to determine which of these favored conformations are consistent with the observation by Stipanovic and Stevens[6] of a positive, 175-nm c.d. band. Finally, the conformations that are retained are further examined to establish which are consistent with the observation of a negative, 185-nm c.d. band at the onset of gelation[6].

RESULTS AND DISCUSSION

The detailed, conformational-energy calculations on gentiobiose and pustulan[4] were initially carried out to shed light on the ability of pustulan to gel with relative ease[6]. This behavior is in contrast to that of dextran, the (1→6)-α-D-glucan, for which gelation has not been observed. The results of the conformational-energy calculations indicated that, although a great deal of flexibility is conferred on the polysaccharide chain by the (1→6) linkage, specific energy minima occur with significant barriers separating them[4]. A comparative study of dextran[7] indicated that the barriers separating the energy minima are smaller for dextran than for pustulan.

Bluhm *et al.*[5] also carried out energy calculations for gentiobiose, with results qualitatively similar to our own. Table I shows the favored conformations found by us[4] and by Bluhm *et al.*[5]. Only the more favorable *gg* and *gt* orientations of the 6-methylene group are included.

We first determine which of these conformations are compatible with the positive, 175-nm c.d. band[6] observed. The quadrant rule which we apply has recently been described in detail[2]; here, we only summarize the relevant elements (see Fig. 2). The quadrant rule is applied separately, and independently, to the two oxygen constituents of the acetal group, O-1 and O-5. For each chromophoric

TABLE I

FAVORED CONFORMATIONS FOR GENTIOBIOSE AND PREDICTED C D

ω	Reference 4		Reference 5		Calc 175 nm c d	Calc 185 nm c d.	Relative energy (kcal/mol)	
	φ (degrees)	ψ (degrees)	φ (degrees)	ψ (degrees)			Ref 4	Ref. 5
gt	20	−90	20	−90	+	−	0.00	2 33
	−20	−150	−30	−140	+	−	0.83	2 92
	40	70	30	60	+	+	—	—
	—	—	50	−10	+	+	—	—
	60	180	—	—	+	+	—	—
	—	—	170	−90	+	+	—	—
gg	155	170	170	130	+	−	1 82	4 06
	165	−120	180	−130	+	+	—	(0.00)
	−25	−150	−30	−150	−			
	—	—	60	110	−			
	—	—	80	110	−			
	15	70	20	80	−			
	—	—	50	−20	−			
	55	180	—	—	−			

oxygen atom, the two symmetry planes are the mirror planes of the C–O–C group. With the oxygen atom of the group oriented toward the observer, the upper-right and lower-left quadrants are positive; *i.e.*, oxygen-containing perturbing groups (OH, CH_2OH) close to the chromophore induce positive c.d. Perturbing groups in the other quadrants induce negative c.d.

Application to pustulan indicates that there are only two strong interactions determining the sign of the 175-nm c.d. band, both of which are conformation-dependent. First, the O-5 atom of a given residue and the O-1 atom of the neighboring residue each acts as a strong perturber of the other, giving rise to strong, positive c.d. for 6-CH_2 *gt* isomers and strong negative c.d. for 6-CH_2 *gg* isomers. The sign of the 175-nm c.d. band is thus dependent on ω. Secondly, each O-1 atom is also strongly perturbed by the O-2 atom in the *gauche* position, which contributes positive c.d. when $\phi = 180 \pm 90°$, and negative c.d. when $\phi = 0 \pm 90°$. The sign of the 175-nm c.d. band is also thereby strongly dependent on φ.

These two interactions contribute additively to the 175-nm c.d. band intensity. We can conclude that all orientations for which (ϕ, ω) = (180 ±90°, *gt*) are expected to display positive c.d. at that wavelength. The relative importance of

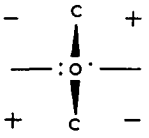

Fig 2. The quadrant rule for the 175-nm circular dichroism band observed in unsubstituted saccharides (ref. 2) The rule is applied separately and independently to the C-1–O-5–C-5 and the C-1–O-1–C-6′ chromophores

the two interactions cannot be established by the quadrant rule alone, so we must also conclude that orientations of the type (ϕ, ω) = (0 $\pm$90°, *gt*) may exhibit positive c.d. at 175 nm, *i e.*, if the O-5–O-1 interaction is stronger than the O-1–O-2 interaction. Similarly, orientations of the type (ϕ, ω) = (180 $\pm$90°, *gg*) will exhibit positive, 175-nm c.d. if the reverse is true. In summary, this step of screening still allows all *gt* isomers and also those *gg* isomers for which ϕ = 180 $\pm$90°. The putative orientations for which ω = *gg* and ϕ = 0 $\pm$90° must be excluded, because such orientations are expected to display negative, 175-nm c.d. Approximately half of the energetically favored orientations are not compatible with the observed v.u.v. c.d. at 175 nm (see Table I)

The second step of screening consists of examining the remaining local-energy minimum conformations, to determine which are consistent with the observation by Stipanovic and Stevens[6] that, at the onset of gelation, pustulan displays a strong, negative c.d. band near 185 nm. This low-energy, c.d band for D-glucans has been assigned by us[3] to an n-σ^* transition on the linkage oxygen atom. We have developed a quantum-mechanical model for calculation of its intensity, using a random-phase approximation, and have published c.d. contours for that transition as a function of conformation. Referring to those c.d contours[3] and to additional calculations performed for the present purpose, we conclude that the only orientations retained by the first step of screening and also consistent with the observed, negative, 185-nm c.d. band are: (ϕ, ψ, ω) = (20°, −90°, *gt*), (ϕ, ψ, ω), = (−20°, −150°, *gt*), and (ϕ, ψ, ω) = (155°, 170°, *gg*) (see Table I).

In our own energy calculations[4] and those of Bluhm *et al.*[5], the first two orientations (*gt*) are approximately 1–2 kcal/mol more stable than the *gg* orientation (see Table I), partly because of a close contact, in the *gg* orientation, between the 6-methylene group and the axial H-2 atom. We are thereby led to a preference for the *gt* orientations as best representing the most probable conformations of pustulan in solution. These two *gt* orientations are closely related The values ϕ = 20° and ϕ = −20° represent rotations to either side of ϕ = 0° which alleviate steric overlap of the 6-methylene group and the H-1 atom.

V u.v. c.d. measurements have thus, in this case, allowed a screening of calculated, local-energy minimum conformations which led to a single pair of closely related conformations, including the global minimum-energy conformation calculated by us earlier[4]. The low-energy, helical conformations which we described earlier[4] may have more relevance to the solid state than to the solution state which we have been considering here.

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