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POLYSACCHARIDE CONFORMATION: EFFECT OF SIDE-GROUP GEOMETRY ON FOUR DIEQUATORIALLY (1→4)-LINKED POLYSACCHARIDES

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ABSTRACT

A systematic approach to the stereochemical aspect of the conformational analysis of the polysaccharides cellulose, polyglucuronic acid, mannan, and polymannuronic acid has been undertaken. A more-exact treatment was given of the main side-groups, and differences from the previously used approximate method were found. A comparison of the flexibility of the four polysaccharides indicates that the polyuronic acids are more flexible than the corresponding glycans and that the mannopyranoses are more flexible than the glucopyranoses. The freedom of rotation of the main side-groups in the conformation of lowest overall potential energy was investigated and found to be restricted, but not sufficiently to cause localization.

INTRODUCTION

The technique of conformational analysis for calculating sterically allowed conformations is now well established[1]. A natural development was its application to the polysaccharides[2–4] and in particular to cellulose[5] and mannan[6]. A general disadvantage of this conformational procedure concerns the confidence one has in the predicted, likely conformation. This arises because the calculations are usually involved with interactions between successive residues along a polymer chain rather than with interactions between chains. The results may therefore be regarded as unrealistic in the sense that calculations relate to the conformation of a single chain in *infinitely* dilute solution. The technique may, however, be extended to interactions between chains[7,8], but some experimental evidence is usually needed to indicate which directions may most usefully be explored[9].

The results are, perhaps, more valuable when experimental touchstones are available, such as X-ray diffraction data from material in the form of stiff gels[10–12] which are not too far removed from the natural situation. However, conformational analysis is very useful, in a negative sense, since it enables a large number of conformations to be ruled out as sterically impossible. In addition, it enables a comparison to be made of the flexibilities of different, yet related, polysaccharides.

Results will be presented from four diequatorially (1→4)-linked polysaccharides, namely, cellulose, polyglucuronic acid, mannan, and polymannuronic acid. Fig. 1

indicates the differences in molecular structure between these four biopolymers. In presenting our results, we will not be concerned in particular with variations in the conformation of the backbone but with the effect of the substituent side-groups of variable geometry.

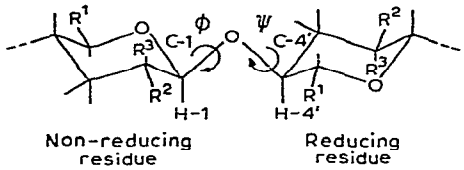


Fig. 1. Schematic diagram of the basic backbone in position $(\phi, \psi) = (0°, 0°)$, showing the sense of rotations ϕ and ψ of reducing residue relative to non-reducing residue. For cellulose, R^1 is CH_2OH, R^2 is OH, R^3 is H; for polyglucuronic acid, R^1 becomes CO_2H. For mannan, R^1 is CH_2OH, R^2 is, H, R^3 is OH; for polymannuronic acid, R^i becomes CO_2H.

METHOD

The usual procedure for polysaccharides is to vary the torsional angles ϕ and ψ (see Fig. 1) about the C-1–O-1 and O-4′–C-4′ glycosidic bonds, respectively, and any conformation having "short" contacts between adjacent residues in the polymer is ruled out in a ϕ, ψ plot[2]. By calculating the potential energy[13] of each stereochemically allowed conformation, the most likely conformation may be obtained by choosing the one having minimum potential energy.

In previous calculations[5,6] on cellulose and mannan, the hydroxymethyl group was approximated to a spherical "blob" centred on C-6. In these original calculations, this was a reasonable approximation and represented the substituent group always moving away to a position of least steric hindrance. However, this situation (as we will show later) is not always stereochemically possible; the substituent groups prefer only certain regions of orientation about the valence bond. The bond angles and bond lengths of these substituent groups are known to the same order of detail as that of the pyranoid ring and may equally well be included in any computer calculations.

Bearing this in mind, we have explored the effect of rotation of the various substituent groups at position 5 by considering rotation about the C-5–C-6 bond. The values of ϕ and ψ have been held constant during this procedure at a number of points in the ϕ, ψ diagram close to the calculated, likely position as found previously[5,6]. By this means, we have compared and contrasted the rotational variations of the hydroxymethyl and carboxyl groups on the basic 1e,4e-linked D-glucopyranose and D-mannopyranose backbones. We have argued that such details may provide information to help explain the differences between these four biopolymers.

The constraints on a regular polysaccharide are directly related to the interactions between successive monomers in the disaccharide unit, the disaccharide unit possessing four principal rotational degrees of freedom. The two pyranoid rings, themselves rigidly held together, are free to rotate about the C-1–O-1 and O-4′–C-4′

glycosidic bonds. In addition, the two substituent side-groups are able to rotate about the C-5–C-6 valence bond in each pyranoid ring. The relative rotation of the hydroxyl groups may be neglected since only the position of the hydrogen atom is affected, and although this is important for hydrogen bonding it is of less consequence in stereochemical calculations involving "hard sphere" criteria. Previous published work[2,5,6] has concentrated on the rotational degrees of freedom about the glycosidic bonds, with neglect of the other two possible rotations. The hydroxymethyl group has been approximated to either a single carbon atom[6] or to a spherical methyl group of various diameters, depending on whether it is in contact with hydrogen, oxygen, or carbon[5]. By a more-complete analysis, the validity of this approximation was tested and extended to a study of the carboxyl substituent group. It is only by considering the details of these substituent groups that cellulose and mannan can be compared with their respective polyuronic acids.

The replacement of the hydroxymethyl group in cellulose by a carboxyl group changes the polymer to polyglucuronic acid. If the hydroxyl groups in the 2 positions are axial rather than equatorial, the related biopolymers mannan and polymannuronic acid are formed.

CALCULATIONS

The geometry of the hydroxymethyl group is shown in Fig. 2. The nomenclature used is in keeping with that proposed at a recent meeting of the American Chemical Society[14]. The bond lengths and angles of the carboxyl group (Fig. 3) were taken from the published crystallographic determination of acetic acid[15].

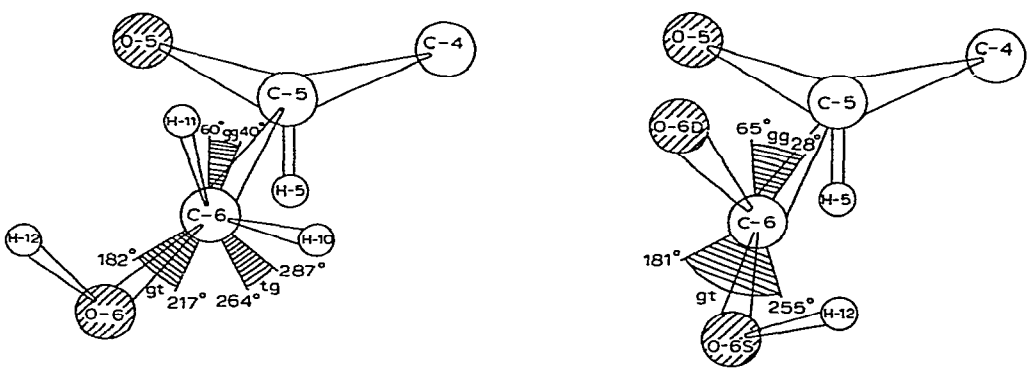


Fig. 2. The hydroxymethyl side-group, showing the three regions (shaded) allowed for O-6 by "hard sphere" criteria. The C–O and C–H distances were taken as 1.43 and 1.07 Å, respectively, and the bond angles were assumed to be tetragonal. The values of χ are shown in degrees.

Fig. 3. The carboxyl side-group, showing the two regions (shaded) allowed for O-6S by "hard sphere" criteria. The C-6–O-6S and C-6–O-6D distances were taken as 1.29 and 1.24 Å, respectively, and the angles C-5–C-6–O-6S and C-5–C-6–O-6D were 116° and 122°.

An initial limiting-procedure was used where the side groups were rotated through one complete revolution of χ and the steric violations between the atoms of the side group and the atoms of the monosaccharide were sought. The interatomic separations used for the "hard sphere" calculations are given in Table I. Fig. 2 also shows that three regions are allowed which correspond to the trans–gauche (tg) notation of Sundaralingam[16]. Since previous conformational calculations for 1e,4e-linked polysaccharides[5,6] have shown that the mean allowable conformation for the backbone is close to a two-fold helix, the hydroxymethyl group on the non-reducing residue, which is far removed from the C-1–O–C-4′ glycosidic linkage, has little effect on these particular calculations. It could therefore be approximated to that of a spherical "blob", the size of a methyl group[5], without affecting the validity of our arguments. The hydroxymethyl group on the reducing residue, on the other hand, is extremely important and must be represented by its individual atoms, the positions of which are shown in Fig. 2.

TABLE I

THE INTERATOMIC SEPARATIONS USED FOR THE "HARD SPHERE" CALCULATIONS

Atomic pair (Å)	*Minimum distance* (Å)	*Marginally allowed distance* (Å)
C–C	3.20	3.00
C–O	2.80	2.70
C–H	2.40	2.20
O–O	2.80	2.70
O–H	2.40	2.20
H–H	2.00	1.90
H–CH_2OH[a]	2.42	2.42
O–CH_2OH[a]	2.90	2.90
C–CH_2OH[a]	3.20	3.20

[a]See ref. 5.

The conformational procedure finally adopted involved selecting values of ϕ and ψ at 10° intervals and calculating the atomic separations with the hydroxymethyl group on the reducing residue represented by a single carbon atom at position 6. The region where no "hard sphere" violation occurred was repeated with ϕ and ψ selected at 2° intervals. If no "hard sphere" violations occurred, the atoms of the complete hydroxymethyl group were introduced at their particular atomic positions and the side group was rotated about C-5–C-6 from the zero position through an angle χ at 1° intervals. For each position of χ, the co-ordinates of O-6, H-10, and H-11 were calculated and also the interatomic separations with all other atoms of the non-reducing residue compared with the minimum allowed "hard sphere" contact-distances. The angle χ was varied until a single position was found for which the triad (ϕ, ψ, χ) produced no stereochemical violation on the "hard sphere" hypothesis, in which case the conformation satisfied the criteria and was deemed "allowed". The

values of χ were preselected to lie in the range of values which offered no stereochemical violation with atoms of the reducing residue.

The variation in the energy contribution from individual atoms in the hydroxymethyl group was assessed as a function of χ in the position (ϕ, ψ) corresponding to the energy minima determined from a potential energy calculation which necessarily used the spherical CH_3 approximation. The programmes were modified to study 1e,4e-linked mannan by changing the atomic co-ordinates of O-2 and H-2. A similar sequence of programmes was written to calculate interatomic separations for rotations of a carboxyl group, the details of which are shown in Fig. 3, so as to enable the corresponding conformational analysis to be carried out for polyglucuronic and polymannuronic acids.

In addition, a programme was written to calculate the van der Waals potential energy for each conformation. The potential energy function used was that of Kitaygorodsky[13] as there is evidence that this form gives slightly more realistic values for carbohydrate structures[6].

DATA

The co-ordinates of the ring atoms were taken from Ramachandran *et al.*[2], after suitable modification from L-glucose to D-glucose. The co-ordinates of the five hydrogen atoms bonded to the ring carbon atoms were calculated on the assumption that they are tetrahedrally bonded. The co-ordinates of the atoms used in the calculations are given in Table II.

The equation used to calculate the potential energy contribution between two unbonded atoms i and j was of the form

$$V_{ij} = 3.5\{-0.04/z^6 + 8.6 \times 10^3 [\exp(-13z)]\},$$

where $z = r_{ij}/r_0$ and r_{ij} is the separation between unbonded atoms i and j. The parameter r_0 depends on the type of the two atoms involved, and their values have been derived by fitting the Kitaygorodsky function to the particular van der Waals potential it represents. The values of r_0 used in the calculation are given in Table III.

RESULTS

It may be seen from Fig. 2 that there are three sterically allowed regions for χ, corresponding to gauche–gauche ($\chi = 40$–$60°$), gauche–trans ($\chi = 182$–$217°$), and trans–gauche ($\chi = 264$–$287°$). In addition, Fig. 3 shows the allowed regions for the carboxyl group. Only two regions are possible which, by comparison with the hydroxymethyl group, are denoted as gauche–gauche ($\chi = 28$–$65°$) and gauche–trans ($\chi = 181$–$255°$). Fig. 4 compares the (ϕ, ψ) plots for cellobiose, using the spherical "blob" approximation, and the results obtained with the hydroxymethyl group. It can be seen that, even with the hydroxymethyl group rotating away from steric interaction, there is a noticeable reduction in the allowed area. The equivalent comparison for the

TABLE II

(*A*) THE CO-ORDINATES OF THE RING ATOMS: D-GLUCOPYRANOSE SYSTEM OF CO-ORDINATES[2]

Atom	*X*	*Y*	*Z*
C-1	1.34	2.08	0.00
C-2	0.05	1.44	−0.47
C-3	0.00	0.00	0.00
C-4	1.24	−0.76	−0.42
C-5	2.50	0.00	0.00
O-5	2.45	1.33	−0.48
O-1	1.43	3.35	−0.53
O-4	1.25	−2.06	0.13
O-2	−1.06	2.17	0.00
O-3	−1.15	−0.62	−0.50
C-6	3.80	−0.64	−0.49

(*B*) THE CO-ORDINATES OF THE HYDROGEN ATOMS BONDED TO THE RING CARBON-ATOMS

Atom	*X*	*Y*	*Z*
H-1	1.34	2.08	1.07
H-4	1.24	−0.76	−1.49
H-5	2.50	0.00	1.07
H-2	0.05	1.44	−1.54
H-3	0.00	0.00	1.07

(*C*) THE CO-ORDINATES OF O-2 AND H-2 FOR THE MANNOSE RESIDUE

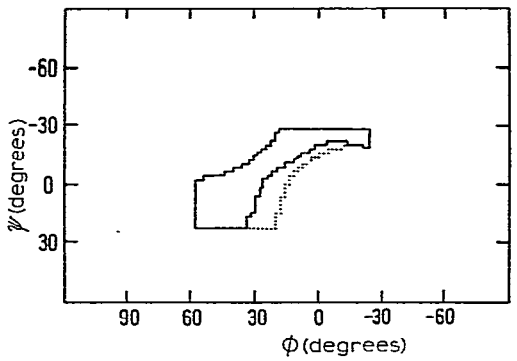
Atom	*X*	*Y*	*Z*
O-2	0.05	1.44	−1.90
H-2	−0.79	1.99	−0.11

TABLE III

VALUES[a] OF THE PARAMETER r_0 IN THE KITAYGORODSKY POTENTIAL-ENERGY FUNCTION

Interacting atoms	r_0 (Å)	*Interacting atoms*	r_0 (Å)
H–H	2.60	O–O	3.33
H–C	3.15	H–CH_3[b]	3.33
H–O	3.00	O–CH_3[b]	3.76
C–C	3.80	C–CH_3[b]	3.92
C–O	3.55		

[a]Use of these numbers gives V_{ij} values in kcal/mole. [b]See ref. 5.



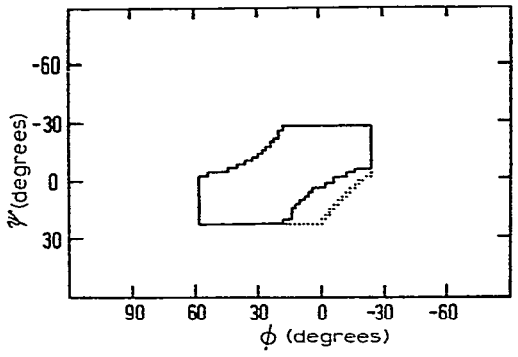


Fig. 4. The (ϕ, ψ) plot for cellobiose, comparing the results obtained with the hydroxymethyl group (enclosed by solid line) with those obtained by using the spherical blob approximation (broken line).

Fig. 5. The (ϕ, ψ) plot for the (1→4)-linked mannose disaccharide, comparing the results obtained with the hydroxymethyl group (enclosed by solid line) with those obtained by using the spherical blob approximation (broken line).

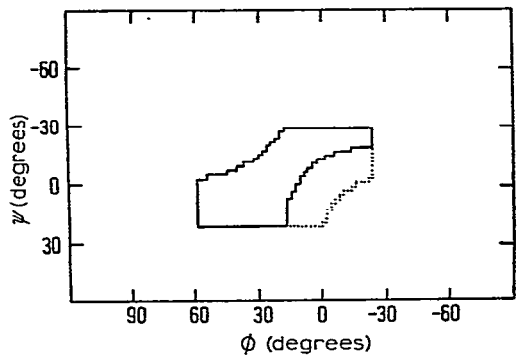
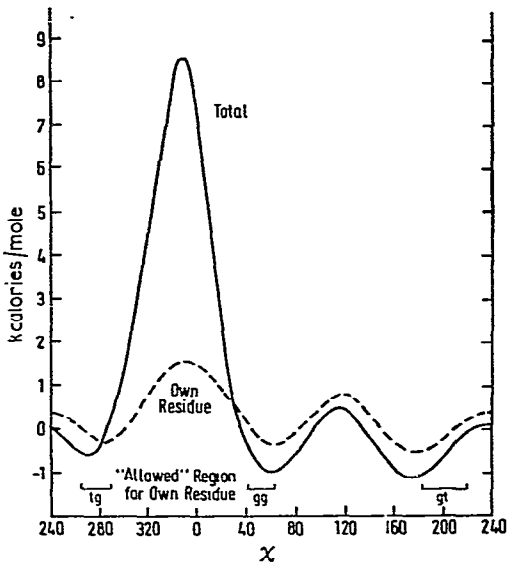


Fig. 6. The (ϕ, ψ) plot for polyglucuronic acid (solid line) and polymannuronic acid (broken line).

mannose disaccharide, shown in Fig. 5, shows again a substantial reduction in possible conformations when the detailed atomic arrangement of the hydroxymethyl group is considered. The results for the uronic acid counterparts of cellobiose and the mannose disaccharide are given in Fig. 6. The potential energy of the hydroxymethyl group rotation in cellobiose is given by Fig. 7. The corresponding results for the carboxyl group of the (1→4)-linked glucuronic acid is shown in Fig. 8.

DISCUSSION

The results show that the conformational flexibility of the diequatorially (1→4)-linked glycans is even more-restricted than was calculated previously. Fig. 4 and 5 show a substantial reduction in the allowed region, when due regard is made for the side-group atoms. It must be remembered that these calculations still allow the side group to rotate to a position of least steric conflict. Further restriction of the side group in any way would result in an even greater drop in the backbone flexibility. In addition to confirming our initial suspicions about the general effect of



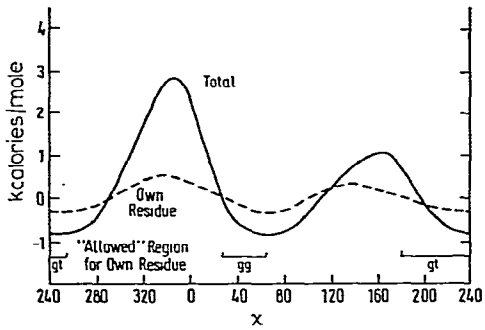


Fig. 7. The potential energy of the hydroxymethyl-group rotation in cellobiose, showing the energy barriers due to its own residue (broken curve) and those due to the disaccharide (solid curve). It should be noted that, as a result of van der Waals attraction between atoms of neighbouring residues, the broken curve is sometimes higher than the solid curve.

Fig. 8. The potential energy of the carboxyl-group rotation in glucuronic acid, showing the energy barriers due to its own residue (broken curve) and those due to the disaccharide (solid curve).

the side groups, some interesting results emerge in a comparison between the glycans and their uronic acid counterparts. It may be seen by comparing Figs. 4 and 5 with Fig. 6 that the uronic acids are more flexible than the corresponding glycans, which relates to the allowed variations in χ shown in Figs. 2 and 3 (total allowed $\chi = 78°$ for hydroxymethyl group and 111° for the carboxyl group). The results also show that mannan and polymannuronic acid are more flexible than the corresponding glucopyranoses.

Cellulose and polyglucuronic acid. — It is puzzling that polyglucuronic acid–one of the most obvious biological polyelectrolytes–does not exist abundantly in Nature. The glucuronic acid unit certainly occurs at alternate sites in the connective tissue glycosaminoglycans[17], but not as a homopolysaccharide in its own right. The conformational analysis results (Figs. 4 and 6) show that it is more flexible than cellulose and therefore cannot be ruled out as stereochemically unfeasible.

Polyglucuronic acid and polymannuronic acid. — A comparison of the two polyuronic acids in Fig. 6 shows that polyglucuronic acid is less flexible than polymannuronic acid. Therefore, although polyglucuronic acid is more flexible than cellulose, it may not be flexible enough to act as a versatile polyelectrolyte.

It may be that a better criterion for polyelectrolytic behaviour is the ability to associate with complex cations, and it has been suggested that this is enhanced by the number of axial substituents[18]. Thus, polyglucuronic acid having no axial substituent groups would be a rather poor polyelectrolyte.

Side-group rotation. — It can be seen from Fig. 7 that the potential-energy barriers for the hydroxymethyl rotation in cellulose are of the order of 1 kcal/mole, relative to its own residue. Thus, when the molecule in solution is flexible, one can imagine these side groups rotating with varying angular velocity as they rise and fall over potential-energy barriers. If the concentration is then increased and the molecular chains are orientated to form a fibre, the potential-energy barrier increases by nearly an order of magnitude. Thus, although the hydroxymethyl group still has some rotational freedom, it may also oscillate on either side of the large potential-energy barrier. It may therefore be imagined that, during fibre preparation, the side groups would not all fall into identical environments, thus leading to disorganized interchain linking. This may well be why cellulose gives such a poor X-ray diffraction pattern.

The corresponding curves for the uronic acids (Fig. 8) show much less of a change and therefore would tend to be more easily crystallized[11].

ACKNOWLEDGMENT

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