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CONFORMATIONAL STUDIES ON β-D-(1→3)-LINKED XYLAN*

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ABSTRACT

The "nonbonded" interaction-energy is computed for a pair of D-xylose residues joined through a β-(1→3) linkages, and also for the helical xylan chain, as a function of (φ, ψ)—the dihedral angles of rotation of the pyranose residues about the inter-residue glycosidic bonds C-1–O and O–C-3′. The conformational map reveals that rotation of the pyranose residues in the polymer chain is highly restricted. The two- and three-strand models proposed for a β-D-(1→3)-linked xylan from X-ray studies have been examined. The individual strands for a double-helical model have lower energies than those in the triple-helical model. However the triple-strand models have the minimum energy if the interstrand interactions are considered. Even though the right- and left-handed, triple-helical conformations have about equal energy (-7.0 kcal.mole^{-1} per residue), the right-handed triple helix is favoured over the left because of the additional possibility in the former of weak hydrogen bonds between strands through water molecules.

INTRODUCTION

An unusual type of xylan wherein the constituent D-xylose residues are β-(1→3)-linked has been identified in a number of seaweeds[1]. This polysaccharide appears to be a skeletal material functioning as cellulose for some of the seaweeds. From X-ray diffraction studies, Frei and Preston[1] suggested for it a double-helical structure consisting of two intertwined helices, each having three xylose residues involved in a fiber repeat of 5.85 Å in the dry state (and 6.12 Å in the wet state). On the other hand, Preston and coworkers[2] have recently proposed a three-strand helical model in the light of helical-diffraction theory and some new X-ray diffraction data, together with i.r. studies. Each strand in this model has six D-xylose residues per turn and a pitch of approximately 18 Å. From the preliminary results of Fourier-transform calculations, these authors tentatively assigned a right-handed helix. Atkins and Parker have put forward further evidence from X-ray studies in favour of the right-

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handed, triple helix[3]. Recently, the calculation of conformational energies has been regarded as a valid approach for determining macromolecular conformations, and many important results have already been obtained in the field of carbohydrate chemistry[4–10]. The present work utilizes this approach with potential functions to calculate the energy of a β-D-(1→3)-linked xylan chain in various conformations and also to examine similarly the possible models proposed from X-ray studies for this molecule.

THEORETICAL CALCULATIONS

A. Fixing of atoms. — It is known experimentally[11–15] and theoretically[15–17] that β-D-xylopyranose normally exists in the *C1* (D) conformation. A section of a xylan chain, consisting of two β-D-xylopyranose residues in the *C1* conformation joined through a (1→3) linkage, is shown in Fig. 1. The various atoms in this molecule

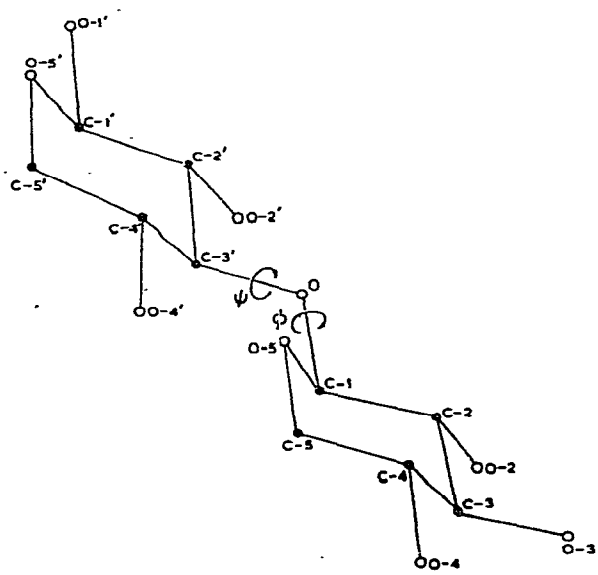

Fig. 1. A pair of β-D-xylose residues joined through a (1→3) linkage. The directions of rotations φ and ψ are marked.

were fixed by using the bond distances and bond angles reported by Ramachandran *et al.*[18]. The angle at the bridge-oxygen atom is assumed to be 116°, since it is the average value observed at the bridge-oxygen atom for other sugars[19,20]. The hydrogen atoms attached to the ring-carbon atoms are not shown in the Fig. 1. The hydroxyl hydrogen atoms were not fixed, since their positions are expected to vary with the environment of the residues. The rotational angles φ and ψ specify the relative orientations of the residues, and their directions are marked in Fig. 1 by arrows. The initial conformation of the residues, $(\varphi, \psi) = (0°, 0°)$, is taken to be the one in which the C-1–H-1 bond is cis to O–C-3′ and C-1–O is cis to the C-3′–H-3′ bond. When viewed from C-1 towards O, a clockwise rotations of the primed xylose residue as

a whole about the C-1–O bond, while the unprimed xylose residue is held stationary, gives an increase in the value of φ. Similarly, when viewed from O towards C-3′, rotation of the primed xylose residue (again in a clockwise direction about the O–C-3′ bond) while the unprimed xylose residue is held stationary, gives an increase in the value of ψ.

B. Potential-energy calculations. — In the present study the "nonbonded" interactions were calculated by using the Kitaigorodsky type of function[21] namely:

$$V(i,j) = 3.5\,(8600\,e^{-13z} - 0.04/z^6) \qquad (1)$$

where $z = r_{ij}/r_0$, r_{ij} is the distance between the interacting atoms i and j, and r_0 is the equilibrium distance between the interacting atoms. The values of r_0 used in the present calculations are the same as those described earlier[4].

The energy of interaction of a pair of D-xylose residues joined through a β-(1→3) linkage, calculated as a function of the dihedral angles (φ, ψ) by using the aforementioned type of function, is shown in Fig. 2. The allowed-region map obtained for xylobiose by using contact-distance criteria[18] is also shown in Fig. 2.

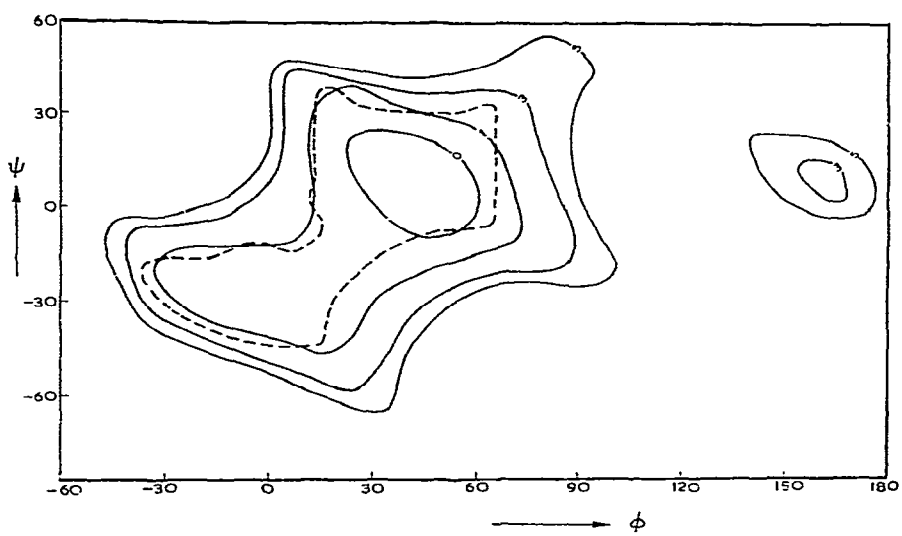

Fig. 2. The energy contours (–) in kcal.mole^{-1} for xylobiose. The extreme-limit contour is indicated (– – –).

The helical parameters, n the number of residues per turn and h the unit translation-height along the helix axis, were calculated for the complete range of φ and ψ from $-180°$ to $+180°$ at intervals of 10°.

The interaction of a residue in a chain having 29 succeeding residues was determined by calculation of the helix energy over a limited range of φ and ψ. The energy contours obtained for the xylan chain are shown in Fig. 3, together with the contours for constant n and h. The chirality of a helix can be represented[8,9] by associating the sign with either n or h, that is, $(n,-h) = (-n, h)$. In the present work,

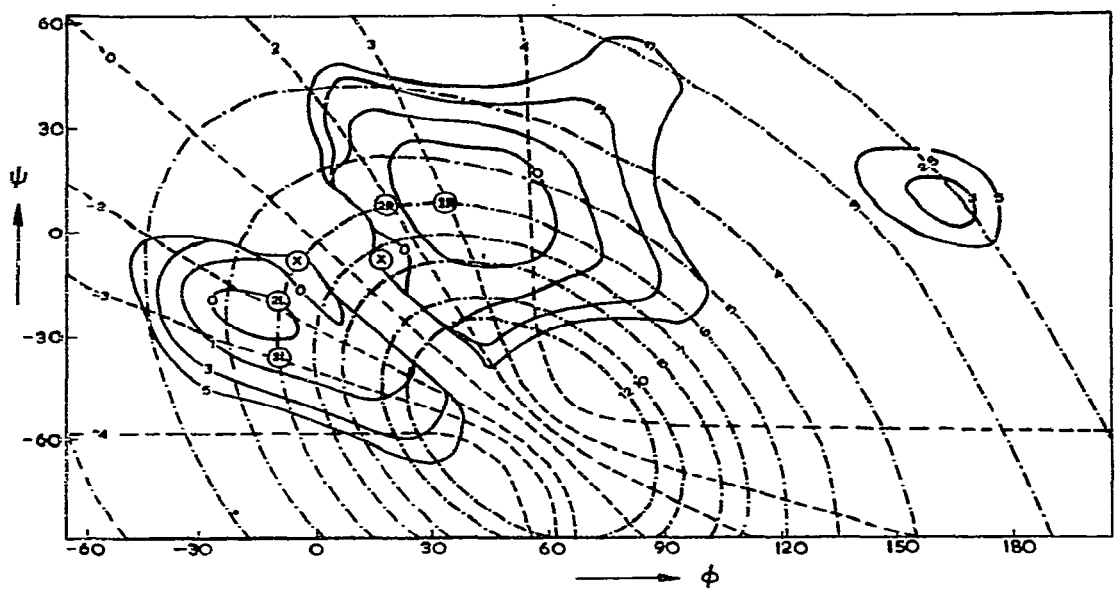

Fig. 3. Energy contours (–) in kcal.mole^{-1} per residue for the single, helical xylan-chain, together with curves of constant n (–.–.–) and h (————); 2R, position of occurrence of the right-handed, double helix of Frei and Preston; 2L, position of occurrence of the left-handed, double helix of Frei and Preston; 3R, position of occurrence of the right-handed, three-strand helix of Atkins *et al.*; 3L, the position of occurence of the left-handed, three-strand helix of Atkins *et al.*; ⊗, the position of occurrence of minimum energy.

therefore, while drawing the contours of curves, for constant n and h, the sign has been associated with h to indicate the chirality of the helix, in order to avoid possible discontinuity in the contours of constant n. Positive and negative values of h indicate right and left chirality, respectively.

C. Hydrogen-bond search. — A search was made in order to determine what hydrogen bonds are possible between adjacent residues. The criteria used for deciding the possibility of hydrogen bonds were that (*a*) the O...O distance should lie between 2.5 and 3.2 Å, and (*b*) the angle at the donor atom should be less than 30°. The results of the hydrogen-bond search are displayed in Fig. 4, and indicate the type of hydrogen bonds possible having lengths within the specified range.

D. Generation of multistrand helices. — As mentioned earlier, the relative orientation of two linked xylose residues can be specified by the dihedral angles (φ, ψ) at the bridge oxygen atom. When the (φ, ψ) values are the same at every linkage the polysaccharide chain adopts a regular, helical conformation. In such a case, the coordinates of each residue can be obtained from those of the preceding one in the chain by rotating the residue through an angle ($360°/n$) about the axis of the helix and translating it through a distance h corresponding to the unit height along the axis of the helix.

In both the double- and triple-helical cases, one of the strands was generated by following the procedure stated above with the appropriate (φ, ψ) values. The

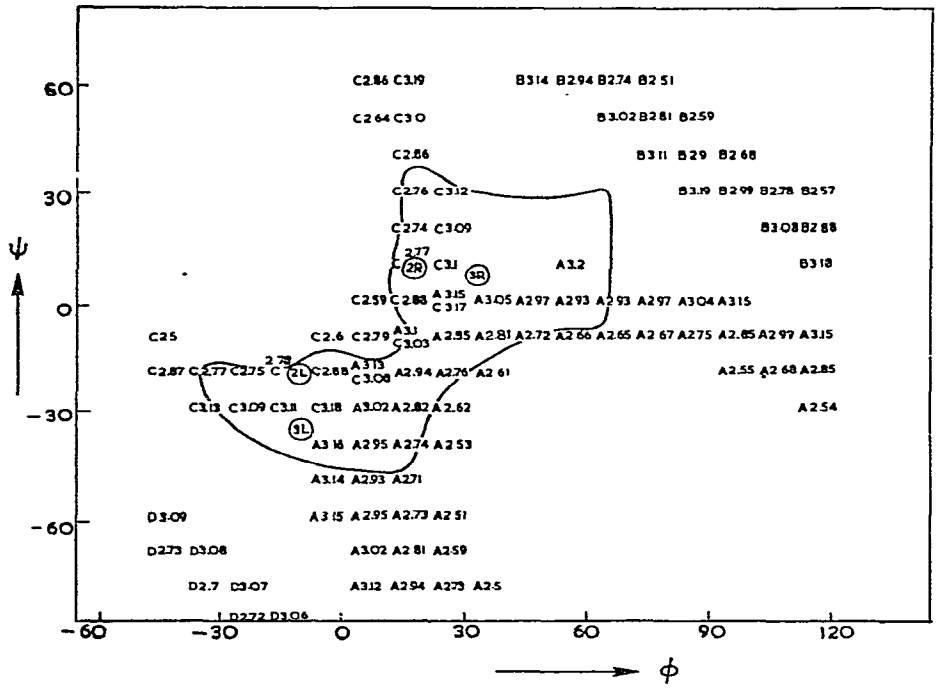

Fig. 4. Extreme-limit contour (–) for a pair of β-D-xylose residues joined through a (1→3) linkage The types of hydrogen bonds are indicated together with the O···O bond lengths; A denotes an O-4′···O-5 type of hydrogen bond; B denotes on O-2′···O-5 type of hydrogen bond; C denotes an O-2···O-2′ type of hydrogen bond; and D denotes an O-4′···O-2 type of hydrogen bond.

second strand of the double helix was generated by rotating the original helix through 180° about the helix axis. Similarly, the second and the third strands in the triple helix were generated by rotating the original (first) strand as a whole about the helix axis through 120 and 240°, respectively. The strands in the above models run in the same direction and along the same helix axis. However, other possible relationships between the chains have not been explored.

DISCUSSION

The small "allowed region" shown in Fig. 2 indicates that the two β-D-xylose residues can be joined without steric strain through a (1→3) linkage, and also indicates that the rotation of these monosaccharide residues is highly restricted. It is seen from Fig. 3 that the energy map of helical xylan chains differs from that of a disaccharide (Fig. 2), mainly in the region near the contour $h = 0$. As the value of $|h|$ decreases, the interactions of atoms between adjacent turns in the helix increases, and hence the region near the contour $h = 0$ is forbidden, as can be seen in Fig. 3. For values of h near zero, the helix would coil back on itself so that atoms of the neighbouring residues in the adjacent turns would be superimposed. Such a situation would give rise to large values of potential energy and hence would be disallowed. There is

only a minor difference between the energy maps for the disaccharide and for the helix in the region remote from the curve of $h = 0$, indicating in practical terms that interactions between the atoms of a residue with neighbouring residues beyond the second are negligible. On either side of the forbidden region, there are two domains where the "non-bonded" interaction-energy passes through a minimum (Fig. 3). Interestingly, these conformational maps have some similarities to that for amylose[9,22], indicating that the chain conformations of β-D-(1→3)- and α-D-(1→4)-linked polysaccharides have some resemblance, as anticipated from model-building studies[23].

Examination of multistrand, helical conformations. — The primary interest in the present calculations is to add some additional information on the models proposed for a β-(1→3)-linked xylan from X-ray studies. Accordingly the two-strand helical model of Frei and Preston and the three-strand helical model of Atkins *et al.*, were examined. Even though recent X-ray studies favour the three-strand helical model[2,3], the two-strand helical model has also been examined in the present paper in order to determine the energetic feasibility of such a structure. Since each strand in the double-helical model of Frei and Preston has six xylose residues per turn and a pitch of 11.7 Å, the positions of occurrence of this model corresponding to $n = 6$ and $h = \pm 1.95$ Å [(18°, 9°) and (−10°, −20°)] are marked in Figs. 3 and 4. Similarly, each strand in the three-strand helical model has six xylose residues per turn and a pitch of 17.6 Å. The positions of occurrence of this model corresponding to $n = 6$ and $h = \pm 2.93$ Å [(33°, 8°) and (−9°, −35°)] are also located in Figs. 3 and 4. Fig. 3 shows that the individual strands, having right and left chiralities occur in both of the models in the regions of lower energy.

It is seen from Fig. 3 that the individual strands in the right-hand, triple-helical structure correspond to the point of lowest nonbonded energy. However, Fig. 4 shows that hydrogen bonds of length about 2.9 Å between contiguous residues in the chain are possible only in either a right- or left-handed, double-helical structure. When the energy due to intra-chain hydrogen bonds is also included, Table I shows

TABLE I

CONFORMATIONAL ENERGY OF A SINGLE STRAND IN VARIOUS MODELS PROPOSED

Model	*Nonbonded energy (kcal.mole^{-1} per residue)*	*Hydrogen-bond energy[a] (kcal.mole^{-1} per residue)*	*Total conformational energy (kcal.mole^{-1} per residue)*
Two-strand, right-handed helix	0.15	−2.2	−2.05
Two-strand, left-handed helix	0.03	−2.2	−2.17
Three-strand, right-handed helix	−0.43	—	−0.43
Three-strand, left-handed helix	0.23	—	0.23

[a]These values were estimated from the curve of hydrogen-bond energy given in Ref. 24.

that the single strand in the left-handed, double-helical conformation has the lowest energy. This evidence alone is not sufficient, however, to establish the favoured

conformation, since the inter-strand interactions have not been considered. Therefore, the "nonbonded" interaction energy was computed for all of the four possible helices by considering the interactions of all of the neighbouring residues in all of the strands of a particular helical conformation.

In calculating the "nonbonded" energy, only the neighbours of an atom that occur in residues separated by up to 7 Å along the axis of the double or triple helix were considered, since atoms that are separated by more than 7 Å make negligible contributions to the nonbonded interaction-energy. Surprisingly, the results of these calculations (Table II) indicate that the triple-helical conformations have the lowest

TABLE II

CONFORMATIONAL ENERGY OF THE VARIOUS HELICAL MODELS PROPOSED

Model	*Nonbonded energy (kcal.mole^{-1} per residue)*	*Hydrogen-bond energy[a] (kcal.mole^{-1} per residue)*		*Total conformational energy (kcal.mole^{-1} per residue)*
		Intra-strand	*Inter-strand*	
Two-strand, right-handed helix	−2.6	−2.2	—	−4.8
Two-strand, left-handed helix	−3.13	−2.2	—	−5.33
Three-strand, right-handed helix	−4.97	—	−2.0	−6.97
Three-strand, left-handed helix	−4.91	—	−2.0	−6.91

[a]See footnote, Table I.

"nonbonded" energy. Moreover, in the triple-helical conformations, the hydroxyl groups at C-2 in the xylose residues point towards the axis of the helix, and the O-2···O-2 distance between neighbouring xylose residues at the same level in the adjacent strands is about 2.98 Å for both the right- and left-handed helices (Fig. 5). On the other hand, no such interstrand hydrogen-bonding is possible in the double-helical conformations. When the energy due to the interchain hydrogen-bonds is also included, the triple-helical conformations have much lower energies than the double-helical conformations (Table II). Interestingly, even though the individual strands in the double-helical conformation have lower energies than the corresponding ones in the triple-helical conformation, consideration of the overall energy of the molecule seems to favour the triple-helical conformation. It thus seems that interstrand interactions play a major role in stabilising the multistrand conformations. Table II shows that the right-handed, triple-helical conformation has about 0.06 kcal.mole^{-1} per residue less energy than the left-handed one. However, this difference is negligibly small and is not sufficient to establish the chirality of the helix. There is evidence that the xylan structure contains one water molecule per xylose residue, even in the dry state[1,2]. Such a water molecule can be placed between the O-4^{I} and O-5^{II} atoms (5.2 Å) of proximate xylose residues in the adjacent chains, but only in the right-handed, helical structure (even though the distance of 5.2 Å is at the upper limit for incorpo-

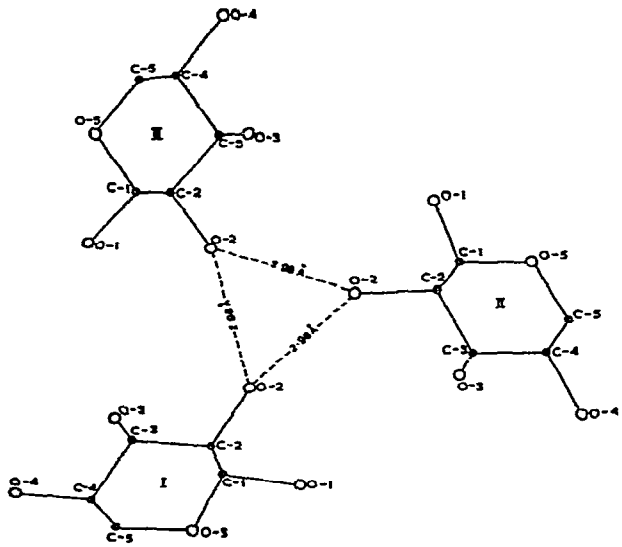

Fig. 5. Projection down the helix axis of a right-handed, three-strand helix, showing the O-2—O-2 types of interstrand hydrogen-bonds. I, II, and III denote the individual helical chains in the structure

rating a water molecule, a slight distortion of the ring might bring the O-4^{I} and O-5^{II} atoms nearer). Such a site is not favourable in the left-handed, helical conformation. Since Atkins *et al.*, considered a right-handed, triple helix the most probable from their X-ray diffraction studies, it appears that the loosely bound water molecules play a major role in deciding the chirality of the helix.

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