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CONFORMATIONAL STUDIES OF POLYSACCHARIDE MULTIPLE HELICES

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ABSTRACT

The possibility of the existence of multiple helices in various homopolysaccharides has been explored by the calculation of conformational-energy contour maps. The structures include homopolymers of D-xylose, D-glucose, D-mannose, and D-galactose, linked α- and β-(1→2), -(1→3), and -(1→4). A number of double parallel-stranded, double antiparallel-stranded, and triple parallel-stranded helices are predicted, all of which are stabilized by interstrand hydrogen-bonds. At least three of the predicted, multiple-helical structures are known to exist. A classification scheme to predict the probabilities of multiple-helix formation is suggested. Possible structure–function relationships of homopolysaccharides are discussed.

INTRODUCTION

Polysaccharides constitute a class of biopolymers whose possible structural variations are almost limitless. In part, this is due to a large number of monosaccharides that serve as monomer residues, and in part, due to their linking to one another in a variety of ways. For example, many of the 12 pyranoside monosaccharides of the D series are well represented in polysaccharides. These sugars polymerize through linkage types 1→2, 1→3, 1→4, (and 1→6 in the case of hexoses), and each type may exist with either an α- or a β-linkage. Recognizing that all of these sugars and linkages could occur in various combinations as linear copolymers, and in addition, as branched polysaccharides, the total number of possible structures may be enormous.

Many such homo- and hetero-polysaccharides are found distributed throughout the animal world, terrestrial and aquatic plants, and bacteria. The diversity of structure of polysaccharides is reflected in their apparent properties and functions, which range from the purely structural (such as cellulose) to food reserve (for instance, starch) to water-retaining gels (such as glycosaminoglycans and polysaccharides of the algae) to the chemical-recognition functions of glycoproteins.

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associated with different cells The structural and gel-forming properties of some of the polysaccharides have made these polymers and their derivatives the basis of important industrial usage

Such diversity of properties is undoubtedly the result of different chemical structure, but the influence of the latter is exerted mainly through its determination of the physical structure, or conformation, of the molecule The key to understanding the functions and structure-properties relationships thus lies not only in the elucidation of the chemical structure, but also in the characterization of the conformation and complete three-dimensional structure This is commonly attempted by X-ray and other diffraction techniques, but the results may be inconclusive because of the well-known difficulties of polymer diffraction-analysis For this and other reasons, predictive analysis of polysaccharide conformation and crystalline structure has become of considerable interest Attempts at these predictions have recently enjoyed remarkable success, both in aiding crystal-structure analysis and in correlating structure and properties For example since the first accurate determination of a polysaccharide crystal structure—that of amylose triacetate, less than ten years ago[1]—through computer-aided conformational analysis, more than ten homopolysaccharides and their derivatives[2] have been similarly characterized with various degrees of refinement The technique has evolved to the point where it can almost compete in precision with the crystal-structure analysis of small molecules However the number of structures is still too small to permit a systematic correlation of the chemical structure of polysaccharides with their conformations or properties Nonetheless, in an attempt to classify the physical structures of polysaccharides, the ranges of stereochemically-allowed conformations of a number of linear homopolysaccharides of different linkages were predicted by Rees and Scott[3], using a simplified form of conformational analysis The resulting probable structures were classified into three types of single-helical conformations identified as A, B, and C Their structures and properties, where known, appeared to be well correlated

However, a number of polysaccharides have been found to crystallize not as single-helical conformations, but as multiple-strand helices For example, $(1\rightarrow3)$-β-D-xylan[4] is a triple, right-handed helix, whereas ι- and κ-carrageenans[5] are double helices, as is agarose[6] From more-recent work, it is highly probable that $(1\rightarrow3)$-β-D-glucans[7,8] are triple helices similar to the corresponding xylan Indications are also strong that A- and B-amylose[9], which are the crystalline components of cereal and tuber starches, respectively, are double helices of as-yet-undetermined characteristics

In view of these findings, it becomes important to analyze systematically the conformations of polysaccharides having all known linkage types, to search out possible multiple-helical conformations and to correlate the predictions with the characteristics of known multiple-helical structures We have begun such a study, and in this communication report initial results for the homopolymers of xylose, glucose, mannose, and galactose

METHOD OF CONFORMATIONAL ANALYSIS

The method is essentially that of the ϕ–ψ type in which the monomeric sugar residue of the homopolymer is kept invariant and all possible chain conformations are created by stepping through 0–360° rotations about the C-1–O_{glyc} bond (ϕ-rotation) and the O_{glyc}–C-x′ bond (ψ-rotation), at suitable intervals In this study, x = 2, 3, and 4, and both α(axial) and β(equatorial) C-1–O_{glyc} bonds were investigated The sugars were assumed to be in the $^4C_1$(D) form with the 5-hydroxymethyl group in the *gg* disposition* The atomic coordinates of each residue were based on the standard residue of Arnott and Scott[11], compiled from crystal structure data on carbohydrates The bond angle at the glycosidic oxygen atom was set at 118° The interval for both ϕ and ψ rotations was usually 10°, which was decreased to 1° in areas of particular interest The $(\phi, \psi) = (0°, 0°)$ position was, by definition, that having the H-1–C-1–O_{glyc}–C-x′–H-x′ bonds in plane and both of the bond sequences H-1–C-1 O_{glyc}–C-x′ and C-1–O_{glyc}–C-x′–H-x′ in *cis* orientation Positive rotations of both ϕ and ψ were clockwise upon observing in the directions C-1 → O_{glyc} and C-x′ → O_{glyc}, respectively, and rotating the farther bond relative to the nearer bond The $(\phi, \psi) = (0°, 0°)$ position for cellobiose is illustrated in Fig 1, which also shows the atomic numbering

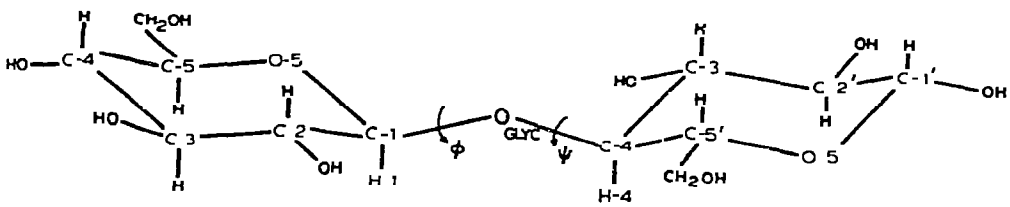


Fig 1 Atom labeling as shown for cellobiose Position shown describes $(\phi, \psi) = (0, 0)$

Multiple-strand helices were created for each ϕ, ψ combination by symmetry operations on the first strand Three types of multiple helices were analyzed—parallel double-helix, antiparallel double-helix, and parallel triple-helix The symmetry operations required to create these multiple helices were, respectively, 180° rotation of the first strand about the helix axis, 180° rotation about an axis normal to the helix axis followed by a one-half turn translation along the helix axis, and ±120° rotation about the helix axis For each conformation, the helix parameters, *n* (number of residues per turn) and *h* (rise per residue in Å along the helix axis), were also calculated Negative *h* values indicated left-handed helices

Other multiple-helical conformations are possible, such as those obtained by sliding individual strands relative to one another along their respective helices, but such structures were not examined It is also possible that some minimum-energy

*See ref 10 for the description of O 6 rotation terminology

TABLE I

COMPARISON OF HELIX PARAMETERS AND MINIMUM ENERGIES OF THE MULTIPLE HELICES PREDICTED FOR VARIOUS HOMOPOLYSACCHARIDES

Polysaccharide	*Type of conformation (Rees and Scott)*	*Type of linkage*	*Double parallel helix*				*Double antiparallel helix*				*Triple helix*			
			n	h	%[a]	*Min E, kcal mole^{-1} residue^{-1}*	n	h	%[a]	*Min E, kcal mole^{-1} residue^{-1}*	n	h	%[a]	*Min E, kcal mole^{-1} residue^{-1}*
D-*Xylan*														
α (1→2)	C	1*a*, 2*e*												
α-(1→3)	A	1*a*, 3*e*	6–7	3	15	−13	6–7	3	1	−12	6–7	3	1	−23
α (1→4)	B	1*a*, 4*e*	5–6	±2–3	4	−19	5–6	±3	1	−18				
β (1→2)	C	1*e*, 2*e*												
β-(1→3)	B	1*e*, 3*e*	5–7	±2–3	25	−20	5–7	±2–3	2	−14	6–7	±3	2	−20
β (1→4)	A	1*e*, 4*e*	5	−3–4	25	−7	5	−3–4	2	−10	−5	25	<1(?)	−1
D *Glucan*														
α (1→2)	C	1*a*, 2*e*												
α (1→3)	A	1*a*, 3*e*	6	2–3	1	−14	6	3	1	−17	6	3	1	−25
α (1→4)	B	1*a*, 4*e*	6	3	<1	−14								
β (1→2)	C	1*e*, 2*e*												
β-(1→3)	B	1*e*, 3*e*	6	±2–3	25	−21	6	±2–3	25	−15	6	±3	2	−21
β-(1→4)	A	1*e*, 4*e*												

TABLE I (*continued*)

Polysaccharide	*Type of conformation (Rees and Scott)*	*Type of linkage*	*Double parallel helix*				*Double antiparallel helix*				*Triple helix*			
			n	h	%[a]	*Min E, kcal mole^{-1} residue^{-1}*	n	h	%[a]	*Min E, kcal mole^{-1} residue^{-1}*	n	h	%[a]	*Min E, kcal mole^{-1} residue^{-1}*
D-*Mannan*														
α (1→2)	C	1*a*, 2*a*												
α (1→3)	A	1*a*, 3*e*					8	3	<1	−3				
α (1→4)	B	1*a*, 4*e*												
β (1→2)	C	1*e*, 2*a*												
β (1→3)	B	1*e*, 3*e*	10–12	±3	1	−7	10–12	±3	1	−8	10–12	±3	1.5	−8
β (1→4)	A	1*e*, 4*e*												
D *Galactan*														
α-(1→2)	C	1*a* 2*e*												
α (1→3)	A	1*a*, 3*e*	7	1–2	<1	−10	7	1–2	<1	−12	7	±1–2	<1	−8
α (1→4)	A	1*a*, 4*a*												
β (1→2)	C	1*e*, 2*e*												
β (1→3)	B	1*e*, 3*e*	5–6	±3	3	−22	5–6	±3	3	−11	6	±2–3	2.5	−22
β (1→4)	B	1*e*, 4*a*	6	−3	3	−20	6	−3	<1	−24				

[a]Percent of total conformations in the complete ϕ, ψ map

conformations were missed because of the relatively large step-size (10°) taken in ϕ and ψ during the search.

The energy of each conformation was approximated by the sum of the nonbonded potential-energy, V_{nb}, calculated by use of a modified Lennard-Jones function:

$$V_{nb} = \sum_i \sum_j (Ar_{ij}^{-12} + Br_{ij}^{-6} + Cr_{ij}^{-3}) \qquad (1)$$

where r_{ij} is the distance between the nonbonded atoms i and j, and the constants A and B are those of Scott and Scheraga[12]. The third term, of magnitude $C = -55$ kcal.Å^3.mole^{-1}, approximates the nondirectional energy of a hydrogen bond in the oxygen–oxygen contact-range of 2.5–3.4 Å. This results in a -4.0 kcal.mole^{-1} minimum in the energy function at 2.8 Å. The total, potential nonbonded-energy was arrived at by pairwise summation over all atoms in one turn of each strand of the helix, relative to a common reference residue in the first strand.

The regions of allowed conformations, namely, all of those conformations having total potential energies equal to or less than 0 kcal.mole^{-1}.residue^{-1}, are indicated in the form of energy-contour maps as a function of the angles ϕ and ψ.

All calculations were performed on a CDC 3200 computer, with a program especially written for this purpose.

RESULTS AND DISCUSSION

The conformational energy-maps for all homopolymers capable of forming multistrand helices are shown in Figs. 2–12, with pertinent information also summarized in Table I. The maps for single helices are not shown, both in the interest of conserving space and because similar maps have, for the most part, previously been published[3,13–15]. Instead, the 0 kcal.mole^{-1}.residue^{-1} contour for single helices has been superimposed on the multiple-helix maps. In some instances, it was observed that the allowed, multiple-helix region did not fall completely within the single-helix, allowed region. This effect was caused by the stability of interstrand hydrogen-bonds[3] adding to the stability of single strands that were only marginally disallowed.

Comparing the conformational features of different polysaccharides by linkage type, it became clear that none of the α- or β-(1→2)-linked polysaccharides could exist as multiple helices. As already noted by Rees and Scott[3], the single helices for these molecules are severely crumpled and experience many nonbonded interactions within the same chain. This factor makes it even more difficult to intertwine individual strands into multiple helices. All such molecules were classified by Rees and Scott[3] as of conformational type C.

The α- and β-(1→3)-linked polysaccharides, for the most part, allow the formation of all types of multiple helices. The exception is (1→3)-α-D-mannan, which allows only a very limited region of double, antiparallel-stranded helices, of relatively high energy. Mannans, in general, do not seem to be able to form stable multiple helices, regardless of linkage [with the possible exception of β-(1→3)], an inability that is caused by the axial hydroxyl group at C-2. It is interesting to compare the

most-probable conformations of multiple helices that are allowed in this linkage type. As shown in Table I, the values of n tend to be in the range of 5 to 7 (with the exception of (1→3)-β-D-mannan, again, for which n is a rather improbable 10–12) and h tends to be in the vicinity of 2–3 Å. The (1→3)-α-D-galactan is an exception here, having h somewhat less, of the order 1–2 Å. This means that, for most of these polysaccharides, a probable molecular repeat per turn should be about 18 Å, which would appear in X-ray diffraction diagrams as either an ~18 Å or ~9 Å fiber repeat for double helices or as an ~18 Å or ~6 Å fiber repeat for triple helices, according to whether the chains have an odd or even value of n, respectively. It is also interesting that all of the α-(1→3)-linked polysaccharides favor right-handed, multiple helices, whereas the β-(1→3) types are equally probable as left- or right-handed conformations.

Within this group of α- and β-(1→3)-linked polysaccharides, (1→3)-β-D-xylan[4] has been determined to exist as a right-handed, triple helix in the crystalline state. The helix parameters for this structure are $n = 6$ and $h = 3.06$ Å, and the three strands are held together by a network of hydrogen bonds between the O-2 atoms of each residue. In the ϕ–ψ map of (1→3)-β-D-xylan, these helix parameters correspond to a position of conformational-energy minimum. The calculations also show that, for this structure, the conformational energy is -20 kcal.mole^{-1}.residue^{-1}, of which -3.8 kcal.mole^{-1}.residue^{-1} results from the interstrand O-2 ... O-2 hydrogen bonds. The same results, indicating triple helices to be the most stable, were obtained in a previous calculation of (1→3)-β-D-xylan conformations[15].

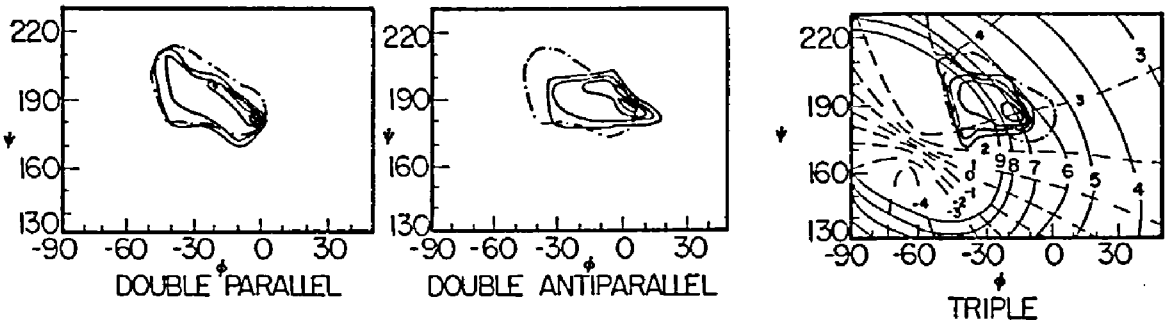


Fig. 2. Conformational-energy maps for multiple-helical (1→3)-α-D-xylans as a function of ϕ, ψ rotations. The energy contours, shown in bold lines, are at intervals of -4 kcal.mole^{-1} residue^{-1} with the highest energy contour equal to 0 kcal.mole^{-1} residue^{-1}. The thin, solid lines denote constant n and the dashed lines denote constant h.

Recent studies have shown that (1→3)-β-D-glucan[7,8] is almost certain to exist in a triple-helical structure similar to that of (1→3)-β-D-xylan. In the ϕ–ψ map of the glucan, there is an energy minimum very near the position found in the corresponding xylan. In addition, an equally low minimum is found for the left-handed structure. The helix parameters at these energy minima are $n = 6$ and $h = \pm 3$. Helix-transform calculations compared with observed X-ray data favor the right-handed structure[8]. The conformational energy for this structure is -21 kcal.mole^{-1}.residue^{-1}, with -3.8 kcal.mole^{-1}.residue^{-1} being due to the same arrangement of hydrogen bonds

as in the xylan The structures of the xylan and the glucan are very similar because the additional hydroxymethyl group of the glucan points away from the other strands of the triple helix, thus exerting no influence on the chain conformation Double-helical regions are allowed for both the (1→3)-β-D-xylan and the corresponding glucan, however, the hydrogen bonding between strands is not so extensive as in the triple-helical structures

The (1→4)-linked polysaccharides are more restricted in multiple-helix formation For example, with the possible exception of (1→4)-β-D-xylan, no triple-stranded helices are allowed Even the latter is questionable, because of unfavorable contacts Only the xylans allow double-helix formation for both α- and β-linkages, which reflects decreased nonbonded interference in this molecule because of the absence of the 5-hydroxymethyl group As previously observed, mannan does not allow any multiple helices with this linkage type, which is a reflection of the importance of the axial hydroxyl group at C-2 Of the glucans and galactans, only the (1→4)-α-D-glucan and (1→4)-β-D-galactan allow double-helix formation This is not surprising because their linkages—1*a* 4*e*, and 1*e*, 4*a*, respectively—result in some-

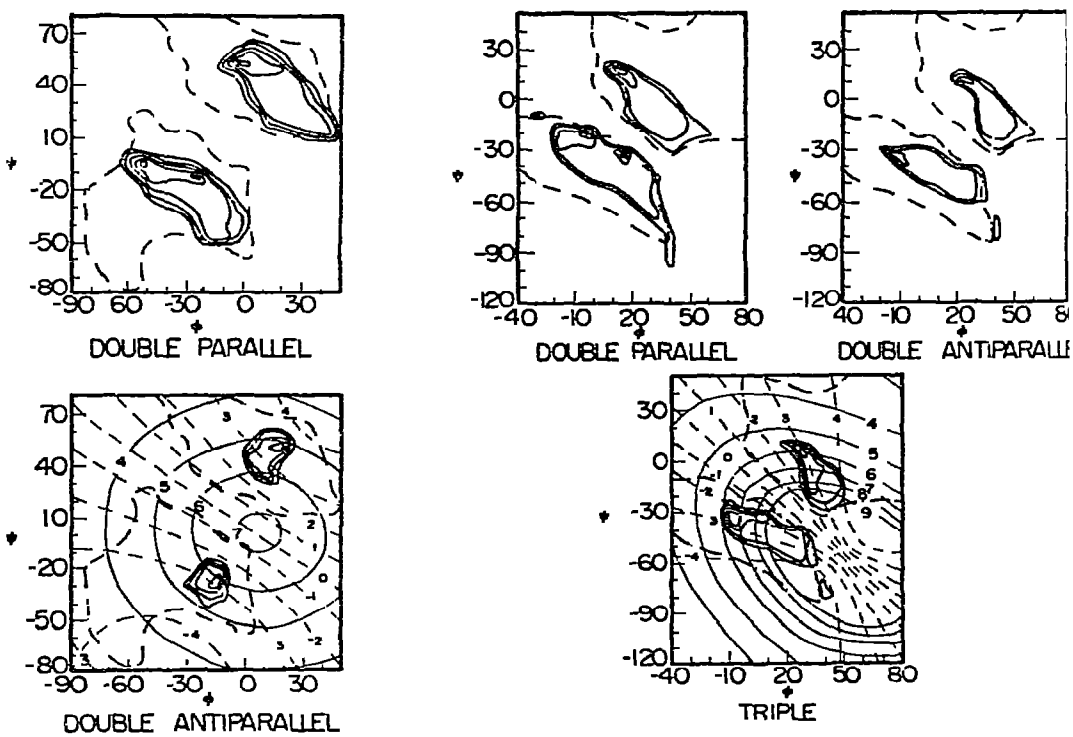


Fig 3 (Left) Conformational-energy maps for multiple-helical (1→4)-α-D-xylans (For further explanation see the caption of Fig 2)

Fig 4 (Right) Conformational-energy maps for multiple helical (1→3)-β-D-xylans (For further explanation see the caption of Fig 2)

what similar chain conformations There does not appear to be a preference for either helix handedness in the (1→4) linked polysaccharides It is interesting, however, that values of n and h center on 5–6 and 3 Å, respectively, as in the (1→3)-linked polysaccharides The general form of the multiple helices in the two classes of (1→3) and (1→4) linkages is thus very similar

Recently, evidence has come to light that A- and B-amylose are both double helical[9] They appear to be of similar molecular conformation, differing only in crystalline packing The double helices have helix parameters of $n = 6$ and $h = 3\ 5$ Å The ϕ–ψ maps of (1→4)-α-D-glucan show a small allowed region for the double, parallel-stranded helix This region surrounds the area having helix parameters $n = 6$ and $h = 3$ Å, and the conformations show no interstrand hydrogen-bonding However, if the glycosidic bond-angle is varied and the 6 hydroxyl group allowed to rotate, the region is enlarged and an interstrand O-6 O-3 hydrogen bond may be formed In this instance, the 5-hydroxymethyl group is in a position between the strands of the helix, and its rotational position is important

Some possible generalizations are emerging from these predictions Rees and Scott[3] predicted that polysaccharides of type B (in their classification) would be the most likely to form multiple helices As is evident from Table I, seven of the eight type-B polysaccharides are indeed able to form multiple helices It is also evident that a further subdivision in this class is possible B_α for α-linked and B_β for β-linked molecules The B_α allow only double-helix formation, whereas B_β generally allow triple-helix formation as well As previously noted, the mannans comprise the exceptions, either by complete inability to form multiple helices or by forming relatively high-energy ones

In addition, five of eight type-A polysaccharides could form multiple helices, although over a very restricted range and generally of only right-handed type and mostly for α-linked molecules The A category could thus be further separated into A_α and A_β subgroups The only A_β allowing multiple helices is (1→4)-β-D-xylan, which allows only left-handed helices that are not very probable in terms of energy The

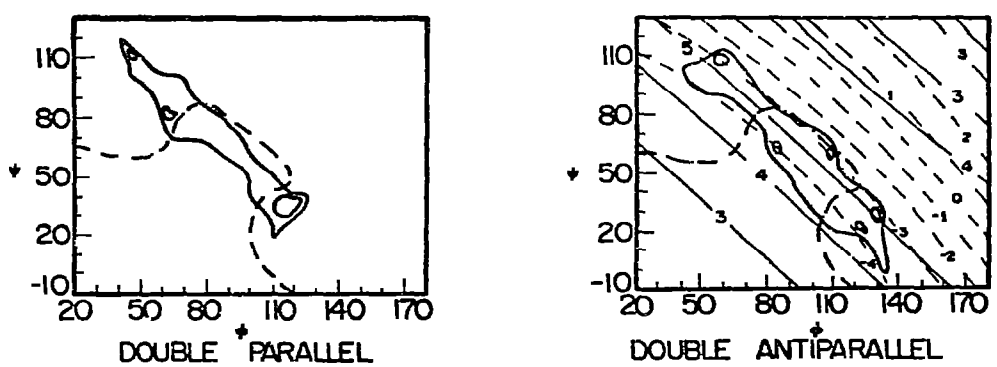


Fig 5 Conformational-energy maps for multiple-helical (1→4)-β-D-xylans (For further explanation see the caption of Fig 2)

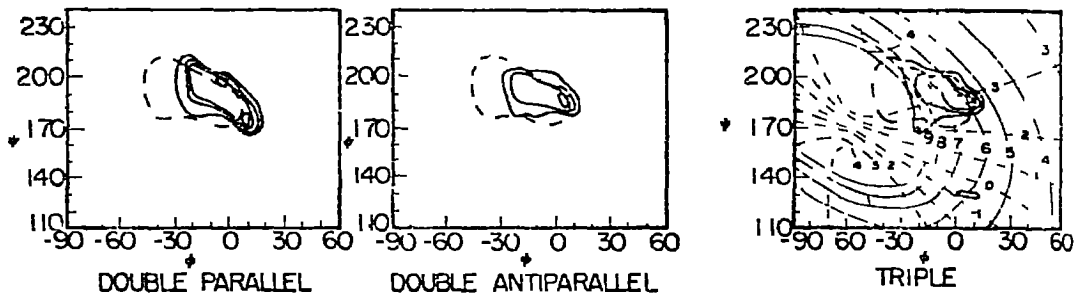


Fig. 6 Conformational-energy maps for multiple-helical (1→3)-α-D-glucans (For further explanation see the caption of Fig 2)

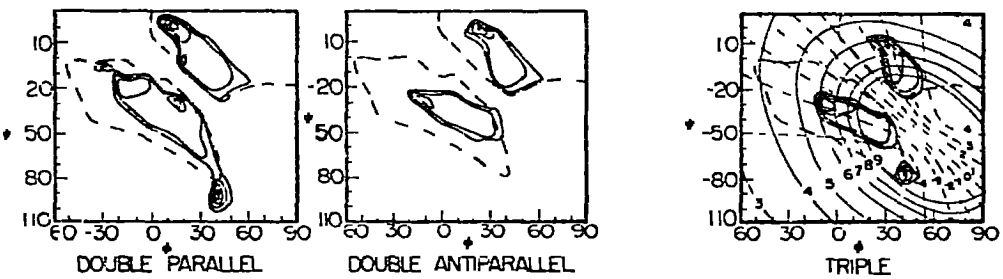


Fig 7 Conformational-energy maps for multiple-helical (1→3)-β-D-glucans (For further explanation see the caption of Fig 2)

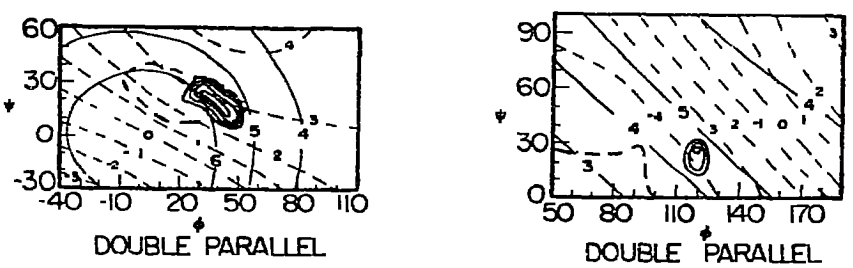


Fig 8 Conformational-energy maps for multiple-helical (1→4)-α-D-glucans (top) and (1→4)-β-D glucans (bottom) (For further explanation see the caption of Fig 2)

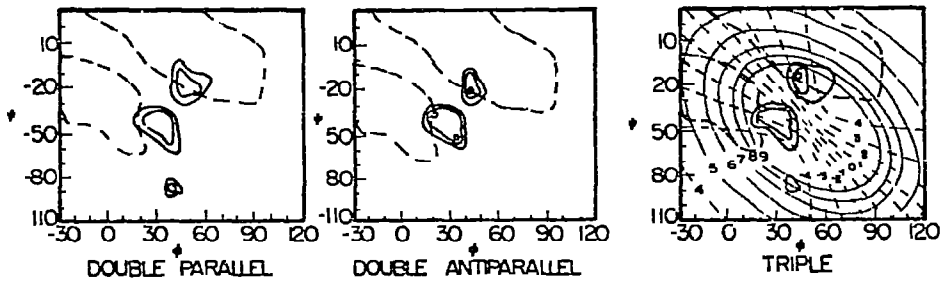


Fig 9 Conformational-energy maps for multiple-helical (1→3)-β-D-mannans (For further explanation see the caption of Fig 2)

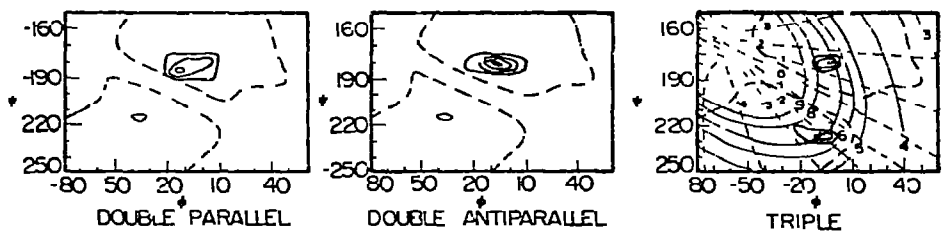
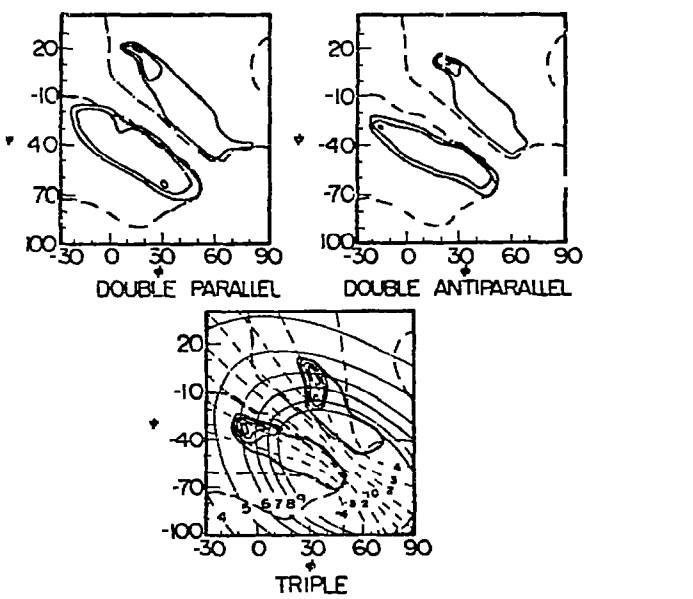


Fig 10 Conformational-energy maps of multiple-helical (1→3)-α-D-galactans (For further explanation see the caption of Fig 2)



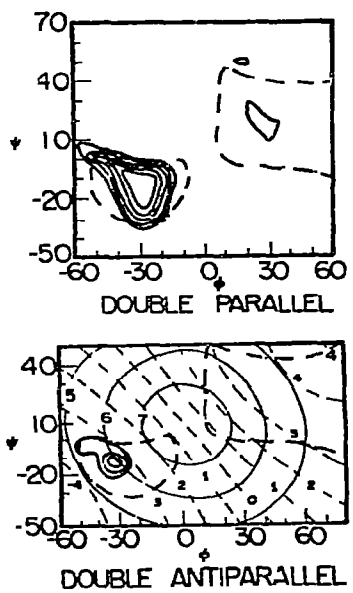


Fig 11 (Left) Conformational-energy maps of multiple-helical (1→3)-β-D-galactans (For further explanation see the caption of Fig 2)

Fig 12 (Right) Conformational-energy maps of multiple-helical (1→4) β-D-galactans (For further explanation see the caption of Fig 2)

exceptions to the foregoing rules are always the xylans and the mannans the first allows more latitude in the types of conformations—undoubtedly because of the absence of the 5-hydroxymethyl group—and the second allows less latitude because of severe restricting effects of the axial hydroxyl group at C-2 These two molecular features should thus be recognized when applying the rules It should also be noted that many of the allowed, multiple-helical conformations are stabilized by interstrand hydrogen-bonds, which often involve the 2-hydroxyl groups

TABLE II

COMPARISON OF TYPE, MULTIPLE HELIX FORMATION, AND FUNCTION FOR KNOWN HOMOPOLYSACCHARIDES

Polysaccharide	*Type*	*Multiple-helix prediction*		*Actual structure*	*Function*
		Probability	*Min energy kcal mole^{-1} residue^{-1}*		
(1→4)-β-D Glucan	A_β	0		Single helix	Structural
(1→4)-β D-Mannan	A_β	0		Single helix	Structural
(1→4)-β-D-Xylan	A_β	Low	−10 (double)	Single helix	Structural
(1→3)-β-D Glucan	B_β	High	−21 (triple)	Triple helix	Reserve, gel former, structural
(1→3) β-D-Xylan	B_β	High	−20 (triple)	Triple helix	Structural, gel former (?)
(1→4)-α-D-Glucan	B_α	Medium*	−14 (double)	Double helix	Reserve, gel former

*High, when O-6 is in other than in *gg* disposition

In Table II, a comparison is made of the structural types, multiple-helix-forming probabilities, the actual structures, and the functions, for all homopolysaccharides at present fully characterized It is clear, within this relatively small sampling, that structural polysaccharides are generally of the A_β type, which either cannot form multiple helices or could form ones that are not well stabilized by hydrogen bonds On the other hand, the reserve polysaccharides are those that are good gel-formers and are of the B_α or B_β type Interestingly, for the B_β type, for which triple-helix formation is possible, the two structures that have been experimentally characterized are both triple helical Similarly, the one B_α type in Table II is double helical in preference to a single helix

CONCLUSIONS

The most interesting finding of this study was the number of polysaccharides that allow some form of multiple-helix formation—11 out of 24, or nearly one half Equally interesting was the fact that the allowed multiple-helix conformations are similar, with little variation in *n* and *h* parameters In some respects, this is not

surprising because there are not too many ways that $^4C_1$(D) monomer residues can form unhindered polymer conformations Moreover, the hydrogen-bond network that stabilizes these helices often involves the 2-hydroxyl groups, thus again introducing similarities

The interstrand hydrogen-bonds appear to be the driving force in the formation of multiple helices Whenever such bonds can be present, all other features remaining constant, the double helix is of lower energy than the single helix, and chances are good that the triple helix is at least as low in energy as the double helix or even lower This seems to be verified by known structures

The lowering of energy through interstrand hydrogen-bond formation in multiple helices may serve as a useful mechanism in stabilizing networks containing large proportions of water Similarly, reserve polysaccharides that need to be reasonably soluble but which must still maintain some dimensional stability—such as starch granules—are ideally structured as multiple helices In contrast, structural polysaccharides, such as cellulose, are best arranged in extended single helices having as much lateral, interhelical hydrogen-bonding as possible

In terms of classifying multiple-helix formers as a function of their chemical structure and conformation, the Rees and Scott[3] scheme still appears valid, but with some modifications In their view, only the type B structures were likely to form multiple helices but, as shown here, some A types also fall into this category At this time, the following classification scheme seems to predict well the probabilities of multiple-helix formation A_α, good, A_β, none, B_α, good for double helix only B_β, good, and C, none Whether this scheme needs to be further modified can only be assessed after more polysaccharide structures have been experimentally characterized

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