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CRYSTALLINE CONFORMATION OF HOMO- AND REGULAR HETEROGLUCAN CHAINS

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ABSTRACT

Crystalline polysaccharides with 1 → 3-β and 1 → 4-β glycosidic linkages are the most prevalent ones in nature. An interpretation of recent X-ray data on 1 → 3-α glucan shows that it has a ribbon-like crystalline conformation similar to cellulose. Comparison of the crystalline conformation of the four principal homoglucans shows that they fall either in the 'ribbon-like' or 'large amplitude' helix class. Heteroglucans with a regular sequence of glucosidic linkages show characteristics of the 'extended conformation' rather than the 'coiled conformation' even when there is 50% of a linkage which in a homoglucan leads to a large amplitude helix. It is concluded that X-ray diffraction analysis fully establishes the hypothesis that the glycosidic linkage type is the determinant of polysaccharide conformation. In this respect, polysaccharides are more like synthetic polymers than proteins or nucleotides; in the latter, it is variation in the substituents which are responsible for the conformational diversity.

1. INTRODUCTION

Of the four possible pyranose homoglucans (four in the α series and four in the β series) those linked in positions 3 and 4 (see Fig. 1) appear to predominate in nature as crystalline substances. Thus cellulose, amylose and curdlan (Harada, 1974) (the latter also called laminaran, lentinan or paramylon depending on the source) corresponding to (1 → 4)-β, (1 → 4)-α and (1 → 3)-β glucan respectively, have crystalline conformations (Gardner & Blackwell, 1974; Sarko & Wu, 1978; Deslandes *et al.*, 1980) corresponding to minimum energy values of the glycosidic dihedral angles Φ, ψ (see Fig. 1). The ribbon-like conformation of the cellulose chain is well known (Hermans, 1948) but the

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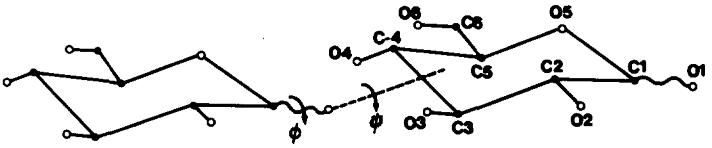

Fig. 1. Schematic diagram showing the eight possibilities of linkage between two glucopyranose units. The anomeric oxygen in position 1 can be linked to the oxygen in positions 2, 3, 4 and 6 of the next unit. On this scheme, Φ and ψ angles are also depicted.

formation of a multiple helical structure by amylose (Sarko & Wu, 1978) and curdlan (Bluhm & Sarko, 1977a, 1977b; Deslandes *et al.*, 1980) is a recently discovered characteristic.

Until recently, experimental evidence concerning the crystalline conformation of (1 → 3)-α-D-glucan has been missing so that verification of the predictions based on minimum energy calculations was not possible. Rees and Scott (1969) calculated the 'average' conformation of the (1 → 3)-α-D-glucan and proposed a ribbon-like structure, similar to cellulose which is known to be fully extended as is also the Klebsiella K-63 bacterial polysaccharide (Atkins *et al.*, 1979). Sathyanarayana & Rao (1972) calculated the isoenergy Φ, ψ map for (1 → 3)-α-D-glucan and confirmed the extended conformation although three energy minima were actually found which left some doubt about the preferred conformation. Bluhm & Sarko (1977a, 1977b) demonstrated that in addition to the single helix a 6_1 double helix or a 6_1 triple helix was theoretically possible.

Recently Ogawa *et al.* (1979) recorded the X-ray fibre diagram of this polysaccharide and reported a fibre repeat of 0·844 nm. The suggested space group was $P2_1$ and a helical conformation with two residues per turn with an advance per monomer of 0·422 nm was proposed.

Computer generated models based on these helical parameters allow one to propose a model for a single chain of this polysaccharide. The calculations were performed with a program written by Zugenmaier & Sarko (1976) which enables the user not only to perform rotations about the 'virtual bond' but also to rotate the 0(6) hydroxyls and to simultaneously modify the ring parameters within constraints applied to bond lengths, bond angles and conformational angles.

2. CONFORMATION OF A SINGLE HELIX

A single helix was generated with a 2_1 symmetry and an advance per monomer of 0·422 nm. The starting glucose residue coordinates were those of Arnott & Scott (1972) corresponding to the $^4C_1$ conformation. During refinement, the glycosidic (bridge) angle, the virtual bond length, all bond lengths, bond angles and conformational angles were varied. Intramolecular hydrogen bonds were defined when the appropriate oxygen–oxygen distance was in the range 0·26–0·30 nm.

The minimum energy conformation of a single chain is illustrated in Fig. 2(a). The 'virtual bond' length (distance between two successive bridge atoms) is 0·425 nm and since the observed advance per monomer is 0·422 nm this means the chain is almost fully extended. Hydrogen bonds with 0(2)–0(4′) distances of 0·288 are predicted for such a chain. The bridge angle is 120° which is slightly higher than the 'standard' value (Arnott & Scott, 1972) of 116°. Three positions (Sarko & Marchessault, 1969)

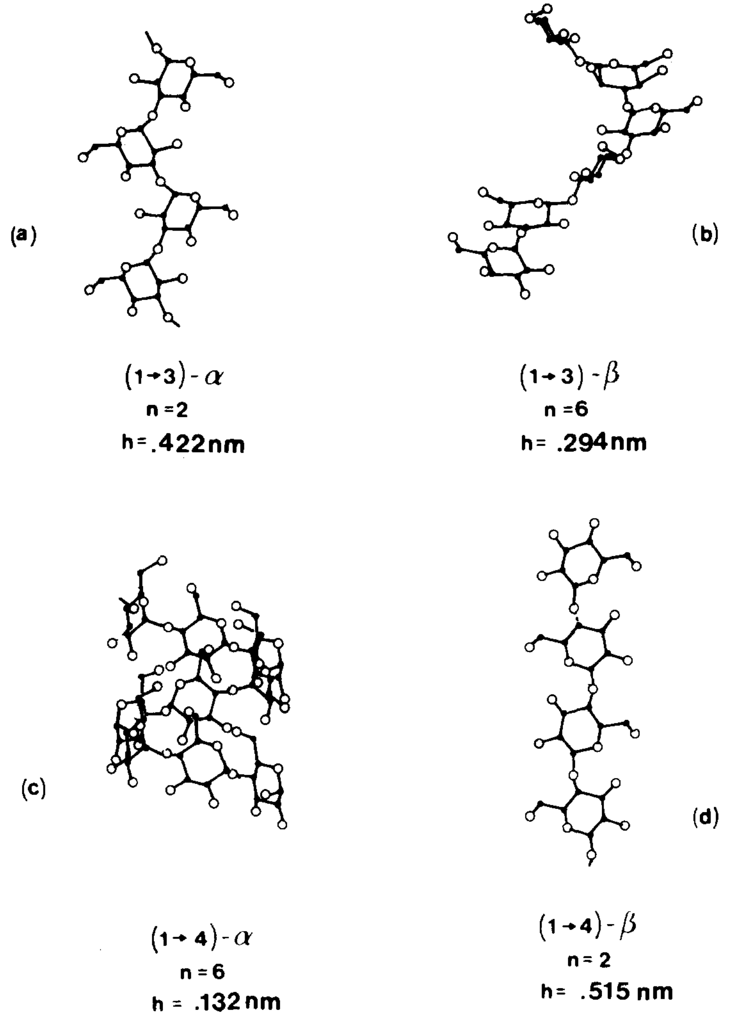

Fig. 2. Crystalline conformation of a single chain for glucans. (a), (1 → 3)-α-D-glucan; (b), (1 → 3)-β-D-glucan (Marchessault *et al.*, 1977; Deslandes *et al.*, 1980); (c), V-amylose (Zugenmaier & Sarko, 1976); (d), cellulose (Liang & Marchessault, 1959). The number of residues per turn *n* and the advance per monomer *h* are given.

of the OH(6) hydroxyl corresponding to *gg*, *gt* and *tg* are possible, since none of them can be ruled out according to the conformational refinement of a single chain.

The extended chain conformation corresponds with one of the energy minima predicted by Sathyanarayana & Rao (1972). The Φ, ψ angles $-5°$ and $-25°$ do not correspond to the global minimum on this map although it is only 4 kJ more than the reported value. According to the map the global minimum is a non-integral helix of 5_2 symmetry. The multiple helix possibility for this polysaccharide as proposed by Bluhm & Sarko (1977a, 1977b) is not in agreement with the observed helical parameters.

Packing of the chains and an X-ray refinement have been performed by Sarko *et al.* (1981) and the final conformation proposed is similar to the model used by the authors although Sarko *et al.* proposed that the twofold symmetry is not located along the macromolecular axis but between the chains.

3. DISCUSSION

Figure 2 shows the four $(1 \rightarrow 3)$ and $(1 \rightarrow 4)$ linked homoglucans in their crystalline conformation (single chain only). Cellulose and $(1 \rightarrow 3)$-α-D-glucan are ribbon-like and nearly fully extended; amylose and $(1 \rightarrow 3)$-β-D-glucan form large amplitude helices, i.e. the virtual bond length is much greater than the observed advance per monomer. In the case of $(1 \rightarrow 3)$-α-D-glucan the advance per monomer is very similar to the virtual bond length and the same is true for cellulose.

The comparison between the two chain pairs is also consistent with the physical properties. Curdlan and amylose in starch are known for their gel forming properties (Harada, 1974; Whistler, 1975); paramylon and starch have a carbon reserve function in plants (Barras & Stone, 1969; Whistler, 1975) which may be related to certain packing and morphological features possible with a large amplitude helical structure (Marchessault & Deslandes, 1979). Although $(1 \rightarrow 3)$-β-D-glucan also plays a structural role when it occurs in the cell walls of yeast (Jelsma & Kreger, 1975) it is probably more of a cell wall matrix substance than anything else. The ability to form multiple helices probably accounts for unusual solubility phenomena such as retrogradation (Whistler, 1975) and irreversible gel formation on heating (Harada, 1974; Marchessault & Deslandes, 1979).

The ribbon-like conformation of $(1 \rightarrow 3)$-α-D-glucan is clearly of general occurrence in nature (Jelsma, 1979). It is so similar to that of cellulose that it is surprising not to find this molecule more universally distributed in the plant world. Packing analyses based on minimum energy consideration only (Deslandes, 1979) and on X-ray diffraction measurements (Sarko *et al.*, 1981) show preference for an antiparallel chain packing (similar to cellulose II) (Stipanovic & Sarko, 1976) which is not in keeping with a morphogenesis involving coordinated synthesis of several chains and simultaneous crystallisation of the structural matter. Thus a natural selection which is based on a biosynthetic mechanism producing adjacent parallel chains could account for the absence of this polysaccharide in the plant world as a structural material.

The structural classification of polysaccharides by Rees (1973) is in agreement with the observed crystalline conformations of homoglucans. He classified the homopolysaccharides into different types according to their 'average' conformation. Cellulose and (1 → 3)-α-D-glucan belong to type 'A', having an extended or ribbon-like chain conformation; amylose and curdlan which form large amplitude helices are classified as type 'B' polysaccharides. The interactions between single chains to form multiple helices, a mechanism which minimises packing energy, were not considered.

Since the glycosidic linkage is the main factor responsible for the chain conformation, at least for unsubstituted homoglucans, it should also be possible to predict the conformation of regular heteroglucans based on their types of linkage. Nigeran, whose crystalline conformation was refined (Perez *et al.*, 1979) would be an ideal polysaccharide to be closely examined. The backbone of this polysaccharide is composed of glucose residues linked by alternating (1 → 3)-α and (1 → 4)-α linkages. The chain (in the dry form) adopts a twofold helix conformation with an advance per

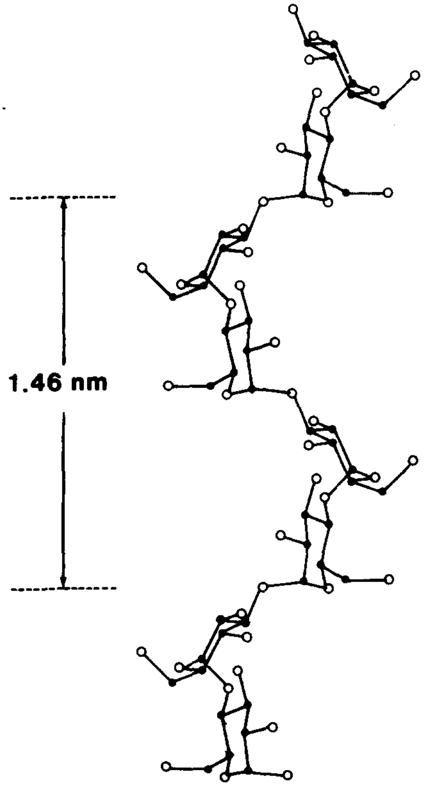

Fig. 3. Crystalline conformation of mycodextran (Perez *et al.*, 1979) in its dry form. The chain is a (1 → 3)-α-maltose and adopts a twofold screw axis with a fibre repeat of 1·46 nm.

monomer of 0·731 nm and is stabilised by an intrachain hydrogen bond between two continuous (1 → 4)-α residues.

Considering the type of linkage, a more or less convoluted chain would be expected: (1 → 4)-α linkage leads to helices, whereas the (1 → 3)-α linkage leads to a ribbon-like chain. Figure 3 shows that the effect of the latter is more important since the nigeran chain conformation corresponds to an extended 'corrugated' ribbon where the kinks in the chain are due to the (1 → 3)-α linkage and the 'stiff' segments are maltose units. The chain can be considered as extended since the advance per monomer (0·731 nm) is only 15% less than the maximum extension (Atkins *et al.*, 1974) of 0·836 nm which is the sum of the virtual bonds of each carbohydrate unit.

Lichenan is another glucan for which the conformation of the chain is known based on conformational analysis and X-ray diffraction (Gaquaire *et al.*, 1975; Tvaroska *et al.*, to be published). This polysaccharide, which can be considered to be a (1 → 3)-β-cellotriose, contains both the (1 → 4)-β linkage and the (1 → 3)-β linkage. The presence of the latter leads to a drastically modified water solubility, since lichenan is water soluble while both cellulose and curdlan are not. Figure 4 shows the chain conformation corresponding to the right-handed threefold helix with an advance per monomer of 1·40 nm. The shape of the helix is reminiscent of the cellulose chain in the cellotriose segments but overall is of a helical character due to the presence of the (1 → 3)-β linkage. Although a large amplitude helix would be expected due to this kind of linkage, the chain is rather extended since the advance per monomer of 1·40 nm is only 10% less than the maximum virtual bond length of 1·55 nm. Clearly, several (1 → 3)-β linkages in succession are required to induce the large amplitude helix.

It would be useful to examine another polysaccharide closely related to glucans the crystalline structure of which is known. Pneumoccocal Type III polysaccharide (Marchessault *et al.*, 1980) is characterised by the presence of alternating (1 → 4)-β and (1 → 3)-β linkages. However, a glucuronic acid residue is present: [(1 → 3)-β-D-Glcp A-(1 → 4)-β-D-Glcp]. The reported conformation of the chain is a slowly winding, almost fully extended helix. Indeed the conformation of Type III Pneumococcal polysaccharide (Fig. 5) is similar to the lichenan conformation (Fig. 4). The virtual bond length of 1·02 nm exceeds the advance per monomer by about 10%. Hyaluronic acid (Winter *et al.*, 1975), a similarly linked polysaccharide, also has a virtual bond length of 1·02 nm and an advance per monomer of 0·95 nm, showing an even more extended crystalline conformation.

4. CONCLUSION

The linkage type is the most important factor in determining the conformation of the chain which in turn will be responsible for the secondary and tertiary structure adopted by the polysaccharide. Thus, the ribbon-like molecule of cellulose can pack readily into dense, rectangular cross-sectioned microfibrils forming an ideal skeleton

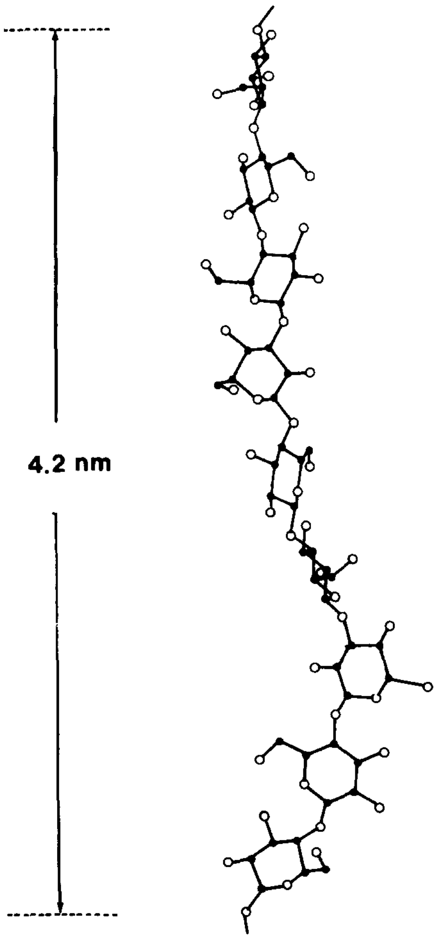

Fig. 4. Crystalline formation of lichenan (Tvaroska *et al.*, to be published). It is a (1 → 3)-β-cellotriose forming a threefold screw axis with a fibre repeat of 4·2 nm.

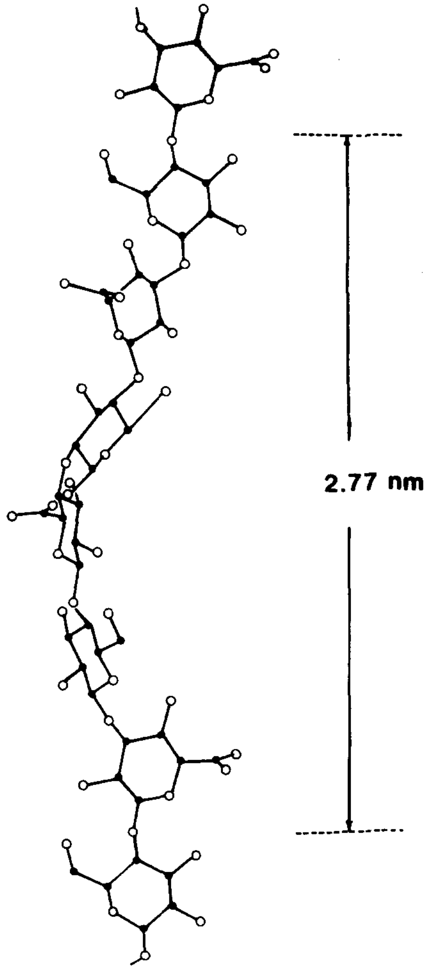

Fig. 5. Crystalline conformation of Pneumococcal Type III polysaccharide (Winter *et al.*, 1975). The backbone is composed of alternating (1 → 3)-β and (1 → 4)-β linkages. The helix is threefold with a fibre repeat of 2·77 nm.

material for cell walls. Similarly, helical conformation and the consequent twinning of helices are dictated by well-defined main chain chemistry. In this respect polysaccharides are more like synthetic polymers than proteins or polynucleotides. In the latter two, it is the variation in the substituents which are responsible for the diversity.

In the heteroglucans the presence of a linkage, whose homoglucan is an extended structure, has a preponderant influence over a linkage which favours a large amplitude helix thus leading to an extended conformation for the heteroglucan. In the Type III Pneumoccocal polysaccharides the Φ, ψ angles at the (1 → 3)-β linkage if repeated continuously would correspond to a sixfold helix and an advance per monomer of 0·40 nm (Sathyanarayana & Rao, 1971) whereas a near extended conformation is found.

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