Carbohydrate Polymers 7 (1987) 133-141

Helical Structure of β(1–3) Xylan and the Water Mediated Hydrogen Bonding Schemes

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(Received 1 July 1986; accepted 11 November 1986)

SUMMARY

A six-fold intertwined triple helical structure for the polysaccharide β(1–3) xylan was generated with the axial advance of 0·306 nm per residue. A stereochemically possible site for the water molecule has been determined and water mediated intrachain and interchain hydrogen bond schemes are possible for the right-handed triple helical structure, whereas only interchain hydrogen bonding appears plausible in the left-handed triple helical structure. The water mediated hydrogen bond is almost linear. X-ray refinement using a Linked-Atom Least-Squares (LALS) procedure has enabled us to determine the orientation of the molecule in the hexagonal unit cell, locate the position of the water molecules and yield a reliability index, R, of 0·35. The refined model in this present study confirms the original chirality of an earlier model but differs in the water mediated hydrogen bonding scheme.

INTRODUCTION

Carbohydrate polymers play a very important role in cell wall functioning of many plant and bacterial systems. Xylan, which is mainly a homopolymer of D-xylose, is mainly distributed on the cell wall of a number of siphoneous green algae. The first comprehensive investigations of the physical structure and morphology of cell wall xylans were reported by Frei & Preston (1961, 1964) using polarised optical microscopy, electron microscopy and X-ray diffraction. Interpretation of the X-ray diffraction results, together with polarised infrared spectroscopic experiments to locate the non-deuterated hydrogen bonds, were reported by

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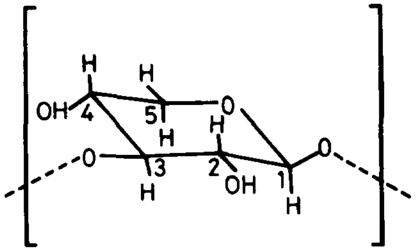
Carbohydrate Polymers 0144-8617/87/$03.50 —



Formula 1. Structure of β(1–3) xylan.

Atkins & Parker (1968, 1969) and Atkins *et al.* (1969). They proposed a molecular rope model for β(1–3) linked xylan consisting of three intertwined polysaccharide chains with the same polarity stabilised by a system of buried interchain hydrogen bonds, to form a triple helix structure. The buried interchain hydrogen bonds occurred between the hydroxyl groups in the 2-position (Formula 1). Interestingly, a number of β(1–3) linked glucans, where the equatorial hydrogen atom in the 5-position is replaced with a hydroxymethyl group, also exhibit X-ray diffraction results with interplanar spacings and features similar to β(1–3) linked xylan and have been interpreted as triple strand molecules (Jelsma & Kreger, 1975; Bluhm & Sarko, 1977; Marchessault *et al.*, 1977; Fulton & Atkins, 1980; Kasai & Harada, 1980) following the reasoning applied to xylan (Atkins *et al.*, 1969; Atkins & Parker, 1969).

Each xylan helix has six xylose units per turn with a pitch of 1·836 nm. A right-handed chirality for the helices was favoured (Atkins & Parker, 1969). The quality of the X-ray diffraction patterns varied with relative humidity. The best patterns were obtained at a relative humidity of 98% and the diffraction signals of this pattern indexed on a hexagonal unit cell with dimensions $a = b = 1{\cdot}54$ nm and c (fibre axis) = 0·612 nm, $\gamma = 120°$. Systematic absences demanded a space group assignment of $P6_3$. The axial advance of each xylose unit in the chain is 1·836/6 = 0·306 nm for the wet sample.

On drying, the values of a and b reduced to 1·32 nm, indicating water was present in the lattice. This was supported by the measured density of 1·57 g/cm^3 which favoured one stoichiometrically bound water molecule per xylose residue. Water mediated hydrogen bond between 04 of residue n (where n is a counting integer) in chain II and 05 of residue $n-2$ in chain I (the rotational angle around the helical axis from chain I to chain II is $-120°$ and chain I to chain III is around $+120°$) was proposed by Atkins and coworkers (Atkins & Parker, 1969; Atkins *et al.*, 1969) based on inspection of molecular models. Sathyanarayana & Rao (1970) calculated the non-bonded energy for both right- and left-handed triple helix ropes and obtained approximately equal energies for both, but favoured the right-handed model because the calculated distance of 0·52 nm between 04 of residue *n* (chain II) and 05 of residue *n* − 2 (chain I) was suitable for the placement of hydrogen bonded water molecule. No suitable site for a water molecule was found in the left-handed structure. Haleem & Parker (1977) calculated the crystallographic reliability index (*R*-factor) for the intertwined right-handed triple helix of xylan by refining the Atkins & Parker (1969) published coordinates of xylan, and obtained an *R*-factor of 0·4. It should be mentioned here that the published coordinates of Atkins & Parker (1969) were based on the laboratory made wire models and therefore less accurate than the coordinates currently available (Arnott & Scott, 1972). In the present work we have generated the stereochemically possible triple helical structures using the Linked-Atom Least-Squares System (LALS) (Smith & Arnott, 1978), refined the structure in the unit cell and determined the possible water mediated hydrogen bonding schemes.

METHOD OF CALCULATION

Standard geometry was used for the xylose residues (Arnott & Scott, 1972) to generate the helical structure of xylan, using the LALS version implemented at this laboratory, running on an IBM 3081 computer. The six-fold right- and left-handed triple helix models with a pitch of 1·836 nm for each helix were generated by varying the glycosidic torsion angles (ϕ, ψ) with minimum constraints (ϕ and ψ are as defined by Sathyanarayana & Rao (1970)). Using the hydrogen bond criteria, an attempt has been made to position the water molecule in the generated triple helical structures. For calculating the crystallographic *R*-factor for the generated helices, the intensity data measured by Haleem (1971) were taken. The X-ray calculations were performed both without the water molecule and with positioning the water molecule in the hydrogen-bonding position. The *R*-factor calculation was obtained for the various orientations of the molecule achieved by rotating the molecule around the crystallographic axis (called the orientation angle, denoted by θ).

RESULTS AND DISCUSSION

The favoured glycosidic torsion angles (ϕ, ψ) generated for the right-handed triple helix of xylan are 34° and 10°, respectively, whereas they

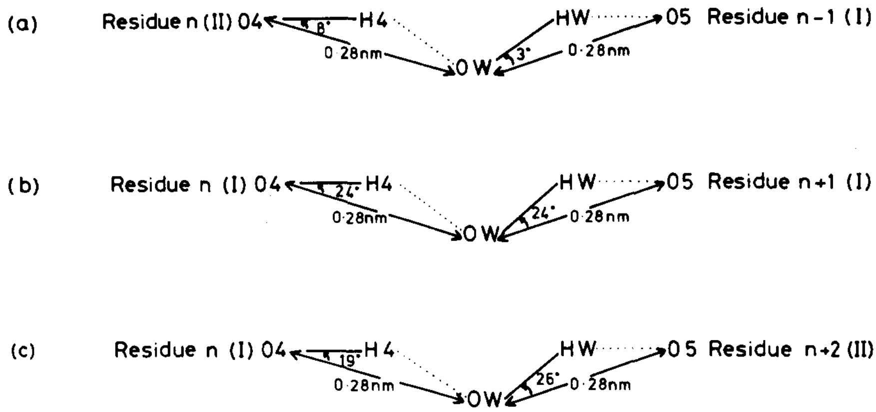


Fig. 1. Schematic representation of the water mediated hydrogen bonding scheme: (a) interchain hydrogen bond in right-handed triple helical structure; (b) intrachain hydrogen bond in right-handed triple helical structure; (c) interchain hydrogen bond in left-handed triple helical structure.

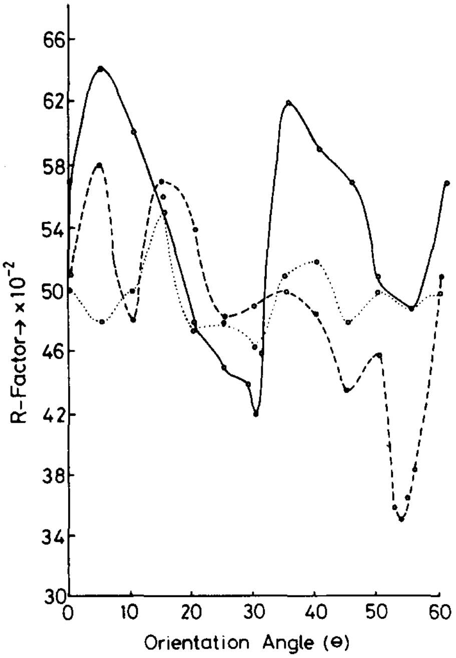


Fig. 2. R–θ curve for the right-hand triple helix: (——) without water molecule; (– – –) water mediated interchain hydrogen bond 04 residue n (II) to 05 residue $n-1$ (I); (·····) water mediated intrachain hydrogen bond 04 residue n (I) to 05 residue $n+1$ (I).

are −10° and −35° respectively, for the left-handed triple helical structure. Both in the right-handed and the left-handed models the hydroxyl at C2 position is pointing towards the helical axis and the distance between them in the different chains is found to be about 0·279 nm, indicating strong interchain hydrogen bonds between the C2 hydroxyls as suggested by Atkins & Parker (1968, 1969) and supported by the infrared spectroscopic results of Atkins *et al.* (1969).

Since xylan contains one water molecule per residue, a water mediated intra- and interchain hydrogen bond search has been carried out for both right-handed and left-handed triple helical structures. Interestingly, water mediated intra- and interchain hydrogen bonds are possible in the right-handed triple helix and only an interchain hydrogen bond is possible in the left-handed triple helix. The hydrogen bonding schemes are shown schematically in Fig. 1. In these positions the water molecule does not cause any steric congestion. A comparison of the hydrogen bonding parameters (Fig. 1) indicates that the water mediated interchain hydrogen bond in the right-handed triple helix (Fig. 1(a)) is strong (as judged by the linearity of the hydrogen bond) when we

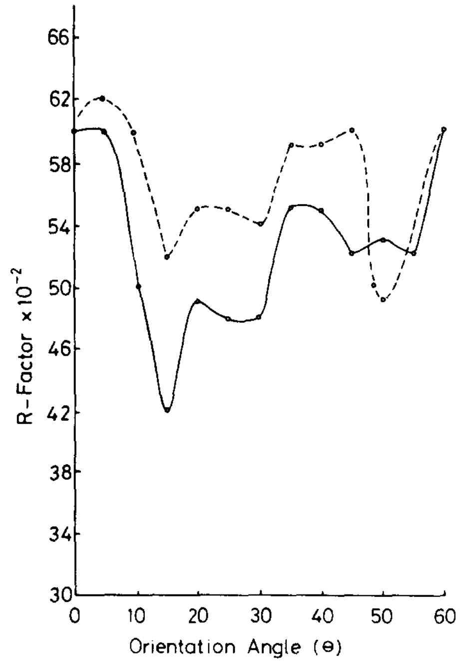


Fig. 3. R–θ curve for left-hand triple helix: (——) without water molecule; (- - -) water mediated interchain hydrogen bond 04 residue n(I) to 05 residue $n+2$ (II).

compare it with the water mediated intrachain hydrogen bond of the right-handed triple helical structure (Fig. 1(b)) and the water mediated interchain hydrogen bond of the left-handed helical structure (Fig. 1(c)). This indicates that β(1–3) xylan favours a right-handed triple helical structure with water mediated interchain hydrogen bond between 04 of residue n (chain II) and 05 of residue $n-1$ (chain I).

The R-factor was calculated for the various orientations (θ) of the molecule in the unit cell and the R–θ curves for the various models are

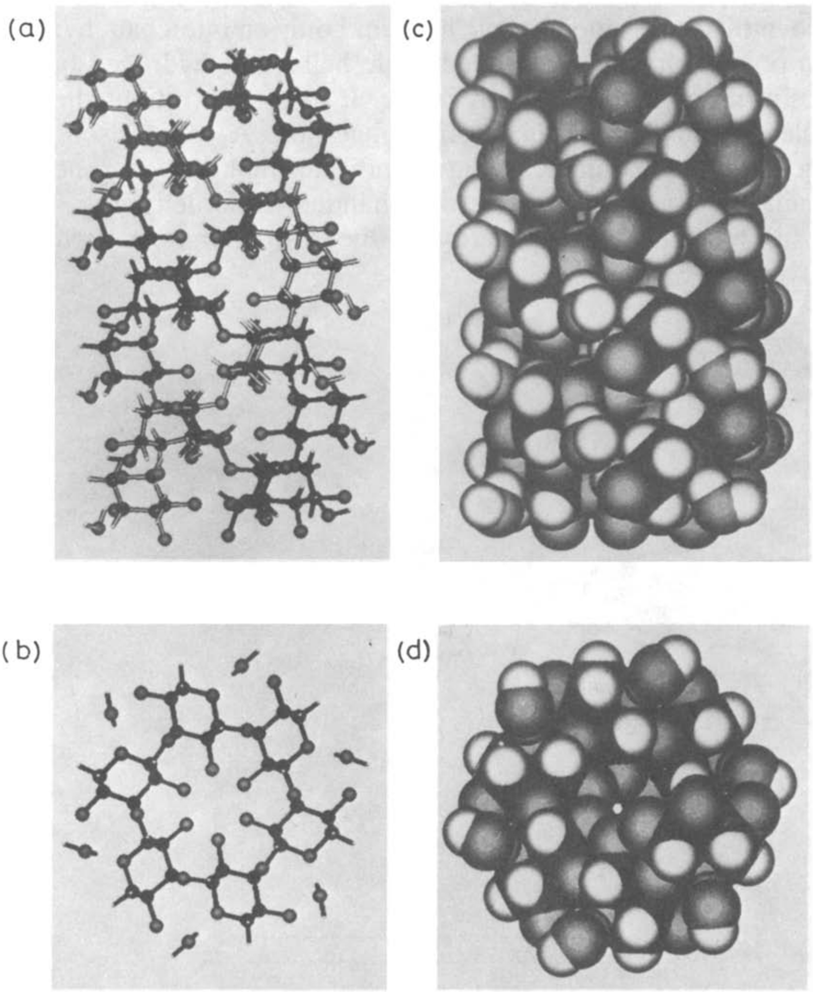


Fig. 4. Projections for the best model of β(1–3) xylan. (a), (b) view perpendicular to the helix axis (c), (d) view along the helix axis.

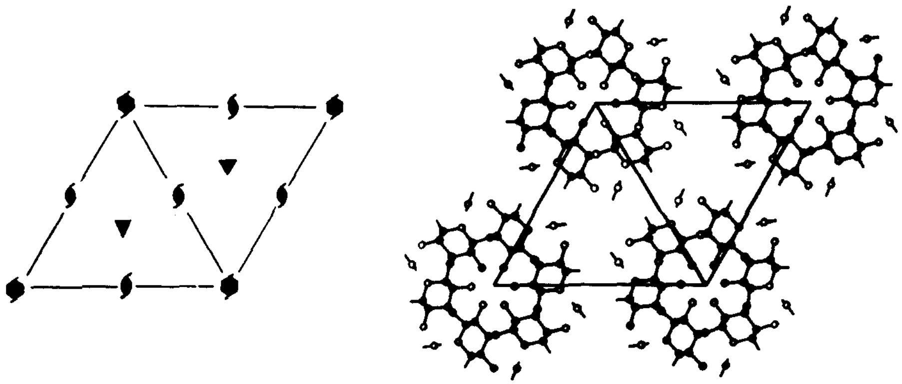


Fig. 5. Spatial orientation of the molecule in the unit cell.

TABLE 1
Observed and Calculated Structure Factor Amplitudes for the Best Model of Xylan

h, k, l	F_o^a	F_c
100	0·633	1·556
200	1·005	0·550
210, 120	0·372	0·011
220	0·410	0·107
310, 130	1·154	0·786
320, 230	0·484	0·555
410, 140	1·266	1·182
330	0·782	0·454
420, 240	0·856	0·218
510, 150	0·894	1·149
520, 250	0·745	0·785
160, 610	0·782	0·579
101	0·782	0·699
211, 121	1·601	0·725
301	0·865	0·635
221	0·596	0·656
311, 131	1·266	1·009
401	1·151	1·104
321, 231	0·670	0·783
411, 141	0·633	0·639
112	1·043	0·348
202	0·521	1·002
212, 122	0·559	0·638
302	0·633	0·642

[a] Observed structure factors taken from Haleem (1971).

plotted in Figs 2 and 3. The R-factor oscillates drastically as θ varies. In the right-hand and left-hand models (continuous curve) the R-factor has a minimum of 0·42. After positioning the water molecule in the right-handed triple helix in the manner depicted in Fig. 1(a), the R-factor reduces to a minimum of 0·35. On the other hand when the water molecule is placed in the position shown in Fig. 1(b), the R-minimum value rises from 0·42 (in the absence of water molecule) to 0·46. When the water molecule is kept in the hydrogen bonding position as shown in Fig. 1(c), in the left-handed triple helix, the R-minimum rises from 0·42 (in the absence of water molecules) to 0·49. X-ray structure analysis shows that the water mediated interchain hydrogen bonded right-handed triple helix has the lowest R-value of 0·35. This is also supported by the evidence that water mediated hydrogen bond is very strong (almost linear) in this model (Fig. 1(a)) compared with that of the other two schemes (Fig. 1(b)) and 1(c)) and is different from the scheme proposed by the earlier workers (Atkins *et al.*, 1969; Atkins & Parker, 1969; Sathyanarayana & Rao, 1970). The projection diagrams corresponding to the best model of xylan are shown in Figs 4 and 5. The calculated structure factors for this model in the minimum R-values position are shown in Table 1.

CONCLUSION

The present studies provide a refined model of $\beta(1-3)$ xylan with the possible water mediated hydrogen bonding schemes. The water mediated interchain hydrogen bonded right-handed triple helical structure was considered to be the best model for xylan, based on the X-ray refinement calculation which leads to the R-value of 0·35. The orientation of the xylan in the unit cell was determined.

ACKNOWLEDGEMENTS

We wish to acknowledge Emeritus Professor R. D. Preston for access to the original X-ray fibre diffraction patterns. We also thank the Agricultural and Food Research Council and the Science and Engineering Research Council for support.

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