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# RIGID BODY REFINEMENT OF WATER MEDIATED TRIPLE HELICAL STRUCTURE OF β-D-1,3 XYLAN FROM *PALMARIA PALMATA* (L.) KUNTZE, RHODOPHYTA)

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**Abstract**

The triple helical structure of β-D-1,3 xylan from the cell wall of *Palmaria palmata* (L.) Kuntze, (Rhodophyta) has been refined using the rigid body refinement technique. Water molecule is also included in the refinement. Using the least square rigid body refinement procedure the structure has been refined to an R-value of 0.338. The agreement between the calculated and observed structure factors is reasonably good. The results indicate that the sugar rings have limited freedom to rotate about 1, 3 glycosidic links and the molecule should be 9% smaller in radius. The position of water molecule is also refined. The loosely bound water follows the helical path. The present results support the view that inter and intra chain hydrogen bonds are possible in the water mediated structures.

## Introduction

The polysaccharides are by far the most abundant organic compounds, constituting more than 90% of the biological macromolecules in nature. The naturally occurring triple helical structures (Atkins & Parker, 1969; Sathanarayana & Rao, 1970) of polysaccharides β-1-3 linked xylan (Feri & Preston, 1961; Iriki & Miwa, 1960) was classified by Frei & Preston (1964) into four families: Byropsidaceae, the Caulerpaceae, the Udotaceae and the Dichotomosiphonaceae. Atkins *et al*., (1968) proposed a noval system of H-bonding in this material. The water soluble xylans have one non-reducing terminal residue of 4-O-methyl-D-glucronic acid for every 11 to 14 xylose units, whereas in the water non-soluble xylans, xylose units can varied from 18 to 65 residues for one non reducing terminal residue of 4-O-methyl-D-glucronic acid (Habibi *et al*., 2002). The chemical structure and interaction of the cell wall polysaccharides from the red edible seaweed *Palmaria palmata* were studied and the results reveal the presence of beta-(1--->4) / beta-1-- >3)-linked D-xylan (Lahaye *et al*., 2003). Haleem & Parker (1977) redetermined the coordinates which represent the standard configuration of glucopyranose ring in the hexagonal unit cell of space group $P6_3$ (Atkins & Parker, 1969). Haleem & Parker (1977) calculated the structure factors of β-D-1, 3 xylan by a new method which was suitable for computer and which avoided Bessel function. These workers refined the structure by least square method. The R-value (Wilson, 1950) and ϕ-value (Arnott & Wonacott, 1966) were found to be 0.41and 4.417 respectively. Haleem & Salma (1985) reduced R-value to 0.37 from 0.41 by number of small modification to the structure including variations in the position of water molecule using observed structure factor of Haleem (1971) Veluraja & Atkins (1987) refined the structure by least square method and determined stereochemically possible site for the water molecule in the right handed triple helical structure.

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The present work describes:

i) Refinement of triple helical structure of β-D-1,3 xylan by least square method similar to other polysaccharides as reported by Akhtar & Haleem (1990) and Haleem & Parker (1977).

ii) Refinement of position of water molecule.
Several new programs were written in basic language and IBM compatible computer was used throughput this work.

**Materials and Methods**

The coordinates representing the standard configuration of glucopyranose ring determined by Haleem (1971), Haleem & Parker (1977) and later refined by Haleem and Salma (1985) were used. The intensity data measured by Haleem (1971) were taken. The intensities for composite eflections were divided in proportion according to calculated values (Haleem & Parker, 1976). The computer method (Haleem & Parker, 1977) was used to calculate structure factors. The method is suitable for computer and which avoids the Bessel function (Haleem & Parker, 1977). The xylan polymer chains are interwind with line symmetry (Haleem & Parker, 1977), each xylan helix has six xylan units per turn with a pitch of 18.36 Å. The chains are arranged in hexagonal lattice with dimensions a = b = 15.4 Å and c = 6.12 Å (fiber axis), β = 120°, with space group as $P6_3$. The axial rise is 3.06 Å (Atkins & Parker, 1969).

Refinement by least square method (Arnott & Wonacott, 1966):

The detailed mathematical calculations are given elsewhere (Akhtar (1988) and Haleem 1971).

The following parameters were used.

P1 = Temperature factor "B".
P2 = Rotation of xylan residue along $O_1$-$O_3$ ($W_4$).
P3 = Change in radial coordinates in reciprocal space (R %).
P4 = Change in real space cylindrical coordinates (θ)
P5 = Change in position of water molecule (Ow- real space cylindrical coordinates)
P6 = Scaling factor (K).

**Results**

The 001 projection of β-D-1,3 xylan unit as determined by Haleem and Salma (1985) and as obtained in the present work is given in Fig. 1. Fig. 2 represents 001 projection of cyclic triad of hydrogen bonds along with position of water molecule after the refinement ($26^{th}$ cycle). This confirms previous findings of Atkins & Parker (1969). The right handed triple helical model of β-D-1,3 xylan is given in Fig. 3, water molecule is also shown in Fig. 3. A comparison of observed factors and calculated structure factors as obtained in the present work is given in Table 1. The ϕ-value (Arnott & Wonacott, 1966) was reduced to 2.572 from 3.447. At this stage R-value (Wilson, 1950) was found to be 0.338.

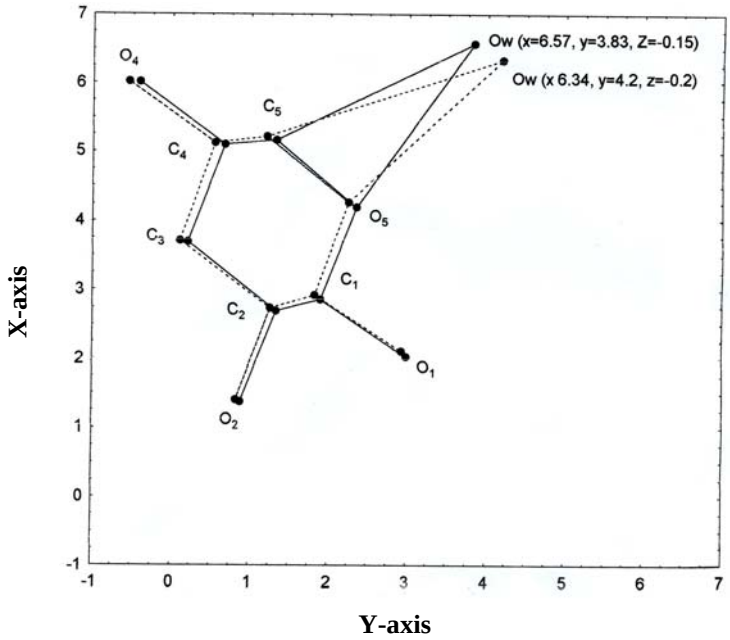


Fig. 1. 001 projection of β-D-1, 3 xylan unit as determined by Haleem & Salma 1985 (shown as dotted line) and as obtained in the present work (shown as continuous line). Ow represent position of water as determined by Haleem & Salma (1985) and as obtained in present work.

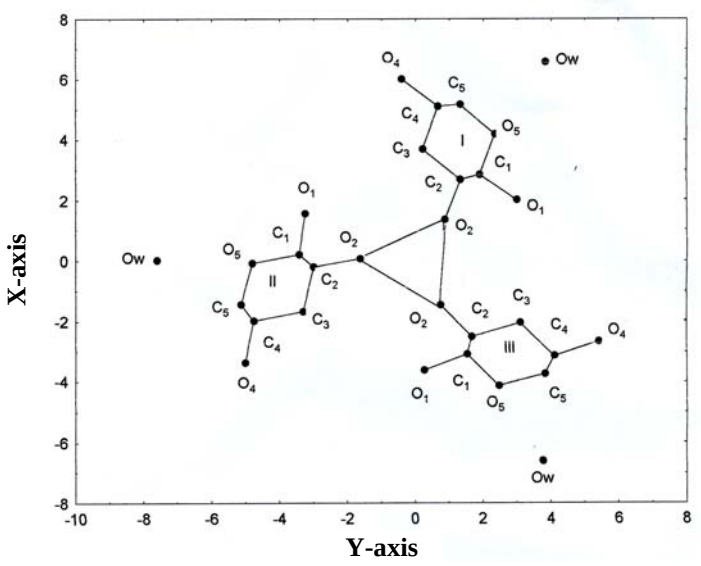


Fig. 2. 001 projection of cyclic triad hydrogen honds after refinement by least square method. The triple helix is stabilized by hydrogen bonds.

**Table 1. Comparison of the observed structure factors (Fo) and calculated structure factors (Fc) as obtained in the present work.**

| h k l | Fc | Fo |
|---|---|---|
| 1 1 0 | 0.4352 | 0.3640 |
| 2 0 0 | 0.9232 | 0.6440 |
| 3 0 0 | 0.3143 | 0.0 |
| 2 2 0 | 0.3463 | 0.4240 |
| 4 0 0 | 0.5396 | 0.0 |
| 5 0 0 | 0.0666 | 0.0 |
| 3 3 0 | 0.2578 | 0.8430 |
| 6 0 0 | 0.1084 | 0.0 |
| 1 0 1 | 0.6352 | 0.6050 |
| 3 0 1 | 0.6825 | 0.6780 |
| 2 2 1 | 0.8509 | 0.5230 |
| 4 0 1 | 0.6897 | 1.3470 |
| 1 0 2 | 0.1627 | 0.0 |
| 1 1 2 | 0.7522 | 1.1830 |
| 2 0 2 | 0.7608 | 0.6520 |
| 3 0 2 | 1.0037 | 0.6650 |
| 2 1 0 | 0.1370 | 0.0849 |
| 1 2 0 | 0.5611 | 0.3478 |
| 3 1 0 | 0.2703 | 0.3801 |
| 1 3 0 | 0.6059 | 0.8520 |
| 3 2 0 | 0.5601 | 0.4165 |
| 2 3 0 | 0.3180 | 0.2364 |
| 4 1 0 | 0.7145 | 1.1239 |
| 1 4 0 | 0.3620 | 0.5694 |
| 4 2 0 | 0.6783 | 0.9318 |
| 2 4 0 | 0.0118 | 0.0163 |
| 5 1 0 | 0.0692 | 0.0846 |
| 1 5 0 | 1.0345 | 1.2651 |
| 4 3 0 | 0.4460 | 0.0 |
| 3 4 0 | 0.0689 | 0.0 |
| 5 2 0 | 0.3795 | 0.4329 |
| 2 5 0 | 0.5950 | 0.6786 |
| 6 1 0 | 0.4485 | 0.3370 |
| 1 6 0 | 1.0270 | 0.7716 |
| 2 1 1 | 1.0733 | 1.2664 |
| 1 2 1 | 0.8602 | 1.0150 |
| 3 1 1 | 0.8384 | 0.7843 |
| 1 3 1 | 1.3529 | 1.2656 |
| 3 2 1 | 0.3756 | 0.4539 |
| 2 3 1 | 0.2655 | 0.3209 |
| 4 1 1 | 0.5110 | 0.4692 |
| 1 4 1 | 0.4528 | 0.4158 |
| 2 1 2 | 0.1412 | 0.1565 |
| 1 2 2 | 0.5217 | 0.5781 |

K-value = 0.016; R-value = 0.338; $\phi$-value = 2.572

hkl = Number of intersections of the particular set of planes with a, b & c axes of a single unit cell.

Fo = Observed structure factors.

Fc = Calculated structure factors.

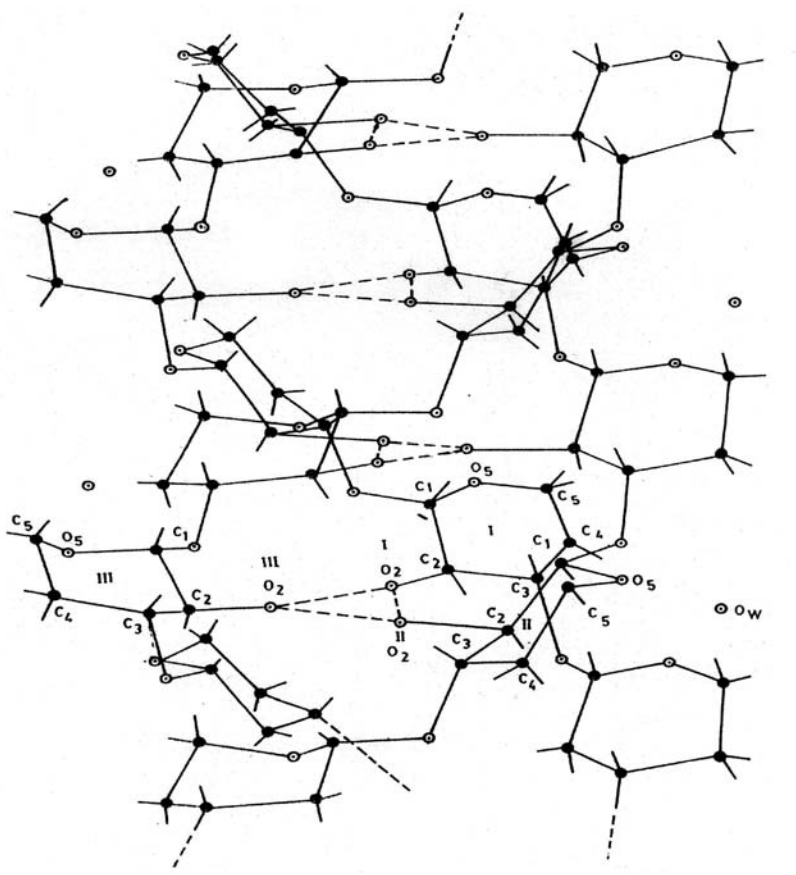


Fig. 3. The right handed triple helical model of β-D-1,3 xylan.

## Discussion

The object in the present calculations is to add informations on the triple helical structure of β-D-1,3 xylan. The geometrical parameters (P2, P3, P4), change in position of water molecule (P5), temperature factor (P1) and scaling factor (P6) were used to refine the structure. The results indicate that the molecule should be 9.1 % smaller in radius (P3). (Haleem & Parker (1977) suggested a value of 8 % for the radial contraction for their structure. A small value of P2 = 0.02 indicates that the sugar rings have limited freedom to rotate about 1,3 glycosidic links. The position of water in the present structure is close to the position determined by Haleem and Salma (1985). The new position of water after the refinement ($26^{th}$ cycle) was found to be r = 7.609, θ = 0.527, Z = -0.147 (r, θ and Z are the cylindrical coordinates of water in real space).

The distance between $O_5$ and $O_W$ were calculated for both structures. The distance between $O_W$ (A) -- $O_5$ (A) $O_W$ (B)-- $O_5$ (B), $C_5$ (A)-- $O_W$ (A), $C_5$ (B)-- $O_W$ (B) is 3.41 Å, 3.35 Å, 3.647 Å and 3.358 Å respectively (A refers to the position of atoms determined by Haleem & Salma (1985) and B refers to the position of atoms obtained in the present

work, $O_W$ represent position of water molecule). Previous workers (Haleem & Parker, 1977; Atkins & Parker 1969) tried to find suitable parameters in order to reduce discrepancies between observed and calculated structure factors.

Haleem & Parker (1977) reported an R-value of 0.41 for their structure. However, Haleem & Salma (1985) adjusted parameters by trial and error method and found an R-value of 0.37 for their structure. In the present work six parameters (P1, P2, P3, P4, P5, P6) were used to reduce triple helical structure R-value to 0.338 from 0.37. The ϕ-value is reduced to 2.572 from 3.447 in 26 cycles. The discrepancies are minimized for the following planes 110, 200, 330, 221, 401, 210, 120, 41, 140, 420, 240 in the present structure. Few observed intensities of the triple helical structures of β-D-1, 3 xylan do not permit to refine the structure further. Only few parameters can be applied to the structure due to lack of side chain. The only hope lies in the refinement with electron diffraction data, provided that a significant increase in the amount of observed data can be realized.

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