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X-Ray study of beijeran sodium salts, a new galacturonic acid-containing exo-polysaccharide

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Abstract

X-Ray fiber diffraction patterns were obtained from oriented films of sodium salts of a new uronic acid-containing polysaccharide (beijeran) both in its native, poly [→ 3)-α-D-GalA-(1 → 3)-β-L-Rha-(1 → 3)-α-D-Glc-*O*6Ac-(1 →], and deacetylated forms. Initially the stretched films of both polysaccharides were amorphous, but the crystallinity was much improved by annealing at high temperature. The deacetylated specimen had higher crystallinity than the native. Both films showed similar X-ray fiber patterns indicating that these polysaccharides had similar unit cell dimensions and that the *O*-acetyl groups in the native beijeran chain did not disturb the regular array in the crystal having space group $P2_1$. All the visible reflections could be indexed in terms of a monoclinic unit cell with dimensions $a = 1.277$, $b = 1.611$, c (fiber axis) = 2.437 nm, and $\gamma = 96.79°$. The fiber axis length and the presence of (002) and (006) reflections indicated that the conformation was made up of two trisaccharide residues, in an extended two-fold helix. © 1997 Elsevier Science Ltd.

Keywords: Beijeran; Chain conformation; Fiber diffraction pattern; Unit cell parameters

1. Introduction

Conformations of uronic acid-containing polysaccharides are receiving much attention because of their potential utilization in the food industry. Those of alginic acids [1–3], pectinic acids [4,5], xanthan [6], and gellan [7,8] have been analyzed using their X-ray fiber diffraction patterns. Alginic acid is a linear copolymer and consists of two fragments, i.e., (1 → 4)-linked α-L-guluronan and (1 → 4)-linked β-D-mannuronan. Pectinic acid contains a linear chain of (1 → 4)-linked α-D-GalA residues, whereas xanthan has trisaccharide side-chains on the backbone (1 → 4)-β-D-glucan chain. The chemical repeating unit of gellan is the tetrasaccharide consisting of two β-D-Glc-*O*6Ac, one β-D-GlcA, and one α-L-Rha residue bonded by 1 → 3 or 1 → 4 linkages, but it has no side-chain [7,8].

Recently, a new uronic acid-containing poly-

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saccharide (beijeran) excreted by a newly isolated bacteria, *Azotobacter beijerinckii* YNM 1, was described. It is a linear polysaccharide consisting of (1 → 3)-linked D-GalA, L-Rha, and D-Glc residues and is anticipated to have potential utilization at food and cosmetic industries [9]. Although it contains an *O*-acetyl group attached at the O-6 position of every D-Glc residue, the backbone chain consists of the following carbohydrate sequence [9]:

→ 3)-α-D-GalA-(1 → 3)-β-L-Rha-(1 → 3)-α-D-Glc-(1 →

Misaki and Oiso [9] have found that the deacetylated beijeran formed an insoluble gel in water in the presence of calcium, whereas the native acetylated beijeran did not, indicating that the *O*-acetyl group inhibits chelation. Similar findings have been reported for gellan [7,8], which, like beijeran, has a tetrasaccharide repeat and similar gelling behavior. We have reported the chain conformation of a calcium salt of the deacetylated beijeran to be an extended two-fold helix based on the X-ray fiber diffraction diagram [10].

Well-defined X-ray fiber diagrams of sodium salts of both native and deacetylated beijerans are described, and a possible single chain conformation is reported based on these patterns.

2. Experimental

Materials and methods.—Beijeran powder was provided by the Development Department of Tayca Co. Osaka, Japan, and prepared as oriented films as described earlier [10]. Sodium salts of both native and deacetylated beijeran films were obtained by preparing each acid form using an acid soln [5:1 (v/v) soln of EtOH and 1 M HCl], followed by neutralization using an NaOH soln [3:1 (v/v) soln of MeOH and 1 M NaOH].

The X-ray diffraction patterns were recorded with a flat-film camera with a Rigaku Geigerflex X-ray diffractometer, using Ni-filtered CuKα radiation generated at 40 kV and 15 mA. The densities of the annealed films of both native and deacetylated beijeran were measured by a flotation method in a carbon tetrachloride–*m*-xylene soln at 30 °C.

3. Result and discussion

The sodium salts of both native acetylated and deacetylated beijeran films did not show any X-ray diffraction spots, indicating that these were amorphous. However, when they were annealed in 75% aqueous isopropyl alcohol at 160 °C in a sealed bomb, the films showed a well-defined fiber pattern at 100% relative humidity, although the native beijeran had less crystallinity than the deacetylated one (Fig. 1). These fiber patterns are very similar, indicating that these samples have similar crystal structures and that the *O*-acetyl groups attached to the backbone chain of the native beijeran are not present in a regular position. Diffuse fiber patterns were obtained when both beijeran films were X-rayed under vacuum. This suggests the presence of water molecules in the crystals.

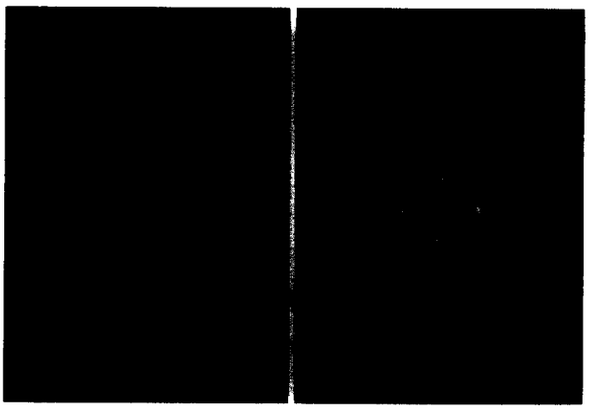

Fig. 1. Fiber diffraction patterns of sodium salts of native (left) and deacetylated (right) beijeran. Fiber axes are vertical.

Table 1
Crystal data for the deacetylated beijeran sodium salt

Crystal system	Monoclinic
Space group	$P2_1$
Lattice parameters	
a (nm)	1.277
b (nm)	1.611
c (fiber axis) (nm)	2.473
γ (degrees)	96.8
Density	
Obsd ($g\,cm^{-3}$)	1.47
Calcd ($g\,cm^{-3}$)	1.47
In the unit cell	
Number of monomer residues	24
Number of the trisaccharide repeating unit	8
Number of water molecules	24
Number of chains	4
Helix parameters [a]	
n	2
h (nm)	1.237

[a] *n*: Number of the trisaccharide repeating unit per turn; *h*: Advanced per the repeating unit along the helix axis.

Table 2
Observed spacings and intensities for deacetylated beijeran sodium salt

			Spacings (nm)		Intensities				Spacings (nm)		Intensities
h	*k*	*l*	Calcd	Obsd	Obsd [a]	*h*	*k*	*l*	Calcd	Obsd	Obsd [a]
1	−1	0	1.056	1.063	M	1	−1	4	0.534	0.532	M
1	1	0	0.941	0.941	VS	1	1	4	0.517		
1	2	0	0.643	0.639	M	2	1	4	0.418	0.417	W
2	0	0	0.634			0	3	4	0.404	0.401	W
0	3	0	0.533	0.532	M	2	−2	4	0.402		
2	−2	0	0.528			1	−3	4	0.395		
1	−1	1	0.972	0.976	S	3	0	4	0.349	0.344	M
1	1	1	0.880	0.882	S	3	−1	4	0.348		
2	1	1	0.553	0.558	VW	0	4	4	0.336		
0	3	1	0.521	0.517	W	3	1	4	0.334		
2	−2	1	0.517			2	−4	4	0.310	0.305	W
1	−3	1	0.503			3	2	4	0.309		
3	0	1	0.417	0.410	M	3	−3	4	0.306		
3	−1	1	0.415			1	−1	5	0.448	0.446	M
1	−4	1	0.390	0.380	M	1	1	5	0.438		
3	−2	1	0.389			1	2	5	0.392	0.388	VW
2	3	1	0.382			2	0	5	0.390		
2	−4	1	0.354	0.350	M	2	−1	5	0.385		
3	2	1	0.353			0	3	5	0.363	0.357	W
3	−3	1	0.349			2	−2	5	0.361		
0	0	2	1.237	1.238	(VS) [b]	1	−3	5	0.356		
0	3	2	0.490	0.491	M	3	0	5	0.321	0.313	W
2	−2	2	0.486			3	−1	5	0.321		
3	0	2	0.400	0.394	M	0	4	5	0.311		
3	−1	2	0.399			3	1	5	0.310		
1	−1	3	0.650	0.648	M	1	−4	5	0.309		
1	−2	3	0.541	0.549	VW	3	−2	5	0.308		
1	2	3	0.507	0.505	VS	2	3	5	0.305		
2	0	3	0.503			0	0	6	0.412	0.414	(M) [b]
0	3	3	0.448	0.450	W	1	−1	6	0.384	0.378	VW
2	−2	3	0.445			1	1	6	0.378		

[a] Abbreviations: VS, very strong; S, strong; M, medium; W, weak; VW, very weak.
[b] (), Meridional reflections.

All 29 visible reflections on the 0th, 1st, 2nd, 3rd, 4th, 5th, and 6th layer-lines (Fig. 1) could be indexed in terms of a monoclinic unit cell with dimensions $a = 1.277$, $b = 1.611$, c (fiber axis) = 2.473 nm, and $\gamma = 96.79°$ (Tables 1 and 2). The volume of this cell and the observed density are in good agreement with the density calculated for 24 monomer residues and 24 water molecules per unit cell for the deacetylated beijeran (Table 2). The unit cell of native beijeran has the same number of monomer residues as that of deacetylated beijeran, but the number of water molecule (16) is different. Thus the calculated density of 1.47 $g\,cm^{-3}$ agrees with the measured density, 1.47 $g\,cm^{-3}$. The presence of (002) and (006) reflections (Table 2) suggests that a two-fold screw axis along the c-axis is present and that a $P2_1$ space group may be assigned. These results indicate that a two-fold helical conformation is made up of two asymmetric units, the trisaccharide residue, and that four beijeran chain segments are included in the unit cell.

The present unit cell of beijeran sodium salts is different from that of calcium salt of deacetylated beijeran, $a = 1.297$, $b = 1.676$, c (fiber axis) = 2.509 nm, and $\gamma = 106.95°$ [10], but the fiber repeats are similar. This indicates that the chain conformations of the sodium salts of native and deacetylated beijerans are similar to that of the calcium salt. As described in the previous paper for the calcium salt [10], we searched an energetically possible two-fold helical conformation of present deacetylated beijeran sodium salt with the restriction of the fiber repeat of 2.473 nm, using the MM3 computer program for the asymmetric trisaccharide conformation, and then, using PS79 [11] for the polysaccharide, assuming that the conformations of both Glc and Gal residues were $^4C_1$ chairs, whereas that of the Rha residue was $^1C_4$. Due to the fact that the asymmetric unit consists of three different monomer units, a great diversity of helical models of beijeran that are energetically possible have been provided. A systematic search of these models and qualitative evaluations of them are under way and will be reported elsewhere. Fig. 2 shows an energetically possible conformation of the beijeran sodium salt under the restriction of fiber repeat. This conformation will be defined by further analysis.

It is interesting to compare the chain conformation of gellan and beijeran, since both polysaccharides have structural and gelling features in common. However, X-ray studies [7,8] indicate that the chain conformation of gellan is an extended left-handed three-fold double helix, whereas beijeran has an extended two-fold helical conformation. The possibility that the latter polysaccharide takes up a double helix will be defined by further analysis. The film of the sodium salt of the deacetylated beijeran did not show any diffraction spot before annealing. It is known that even so-called 'amorphous cellulose' shows some very blurred reflections. The absence of spots was also noticed with the sodium and calcium salts of the deacetylated beijeran [10]. These observations indicate that this polysaccharide can be relatively amorphous, and this property may be important in its applications.

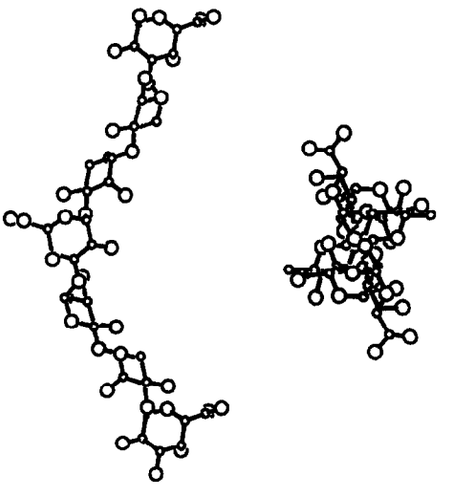

Fig. 2. A possible extended two-fold conformation of deacetylated beijeran sodium salt, projected perpendicular (left) and parallel (right) to the chain axis. Large circles are oxygen and small circles are carbon atoms. All the hydrogen atoms are omitted.

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