Carbohydrate Polymers 3 (1983) 287-297

X-ray Diffraction Data for (1→3)-α-D-Glucan Triacetate

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(Received: 1 November 1982)

ABSTRACT

The crystal structures of (1→3)-α-D-glucan triacetates were studied by X-ray diffraction measurements on fibre diagrams. The oriented films annealed in water at high temperature were of higher crystallinity and occurred as two crystalline polymorphs (GTA I and GTA II) depending on the samples and also the annealing temperature. All reflections in GTA I were indexed with a pseudo-orthorhombic unit cell with a = 1·753, b = 3·018 and c (fibre axis) = 1·205 nm. From the fibre repeat data coupled with the density data and the presence of only the (003) reflection on the meridian, an extended three-fold helical structure was proposed. Although some reflections in GTA II split from the layer lines, the basic unit cell was a monoclinic system with a = 1·685, b = 3·878, c (fibre axis) = 1·210 nm and γ = 112·2°. A similar three-fold structure to GTA I was proposed from the almost identical fibre repeat and the conformational analysis on (1→3)-α-D-glucan. It was concluded that, on acetylation, the D-glucan structure changed from the fully extended two-fold helix to the extended three-fold accompanied by some extent of chain shrinking.

Carbohydrate Polymers 0144-8617/83/$03.00

INTRODUCTION

(1→3)-α-D-Glucan is widely distributed in microorganisms, the cell walls of fungi and yeasts. Crystal structures determined by X-ray diffraction of the D-glucans of fungal cell walls have been extensively investigated by Jelsma (1979) and Jelsma & Kreger (1979, 1980). They found that the fungal D-glucan crystallised in three polymorphic forms, with the native form (polymorph I) appearing to be different from the other two forms (polymorphs II and III) that can be obtained from it. Bacterial (1→3)-α-D-glucans are of interest in connection with dental caries. *Streptococcus mutans* and *S. salivarius* found in human saliva synthesise water-insoluble and sticky polysaccharides from sucrose which are (1→3)-α-D-glucan backbone chains having many short side chains, and cause dental plaques and subsequently dental caries (Ebisu *et al.*, 1974; Ogawa *et al.*, 1979).

Recently we obtained a very good fibre pattern, but of a different polymorph from I–III, from the (1→3)-α-D-glucan prepared from the *S. salivarius* polysaccharide (Ogawa *et al.*, 1979). In order to prepare a well-oriented D-glucan film, however, acetylation of the D-glucan was required. Thereafter the D-glucan triacetate film could be stretched and subsequently deacetylated without any drastic change occurring in it. The resulting polymorph was termed the 'regenerated form' (Ogawa *et al.*, 1981) or polymorph IV (Ogawa *et al.*, 1980). Because of its high quality, the molecular and crystal structure of the polymorph IV could be solved (Ogawa *et al.*, 1981).

Polymorphs of acetyl derivatives of other homoglucans have been studied, e.g. amylose (Sarko, 1966; Sarko & Marchessault, 1966, 1967), cellulose (Chanzy & Roche, 1974; Marchessault & Chanzy, 1977; Stipanovic & Sarko, 1978) and (1→3)-β-D-glucan (Bluhm & Sarko, 1975). In the present paper, well-defined X-ray fibre patterns of (1→3)-α-D-glucan triacetate will be reported, and based on the patterns, we will propose chain conformations of the D-glucan acetate.

EXPERIMENTAL

Materials

Three samples of (1→3)-α-D-glucan prepared from different origins, one from a bacterial extracellular polysaccharide and the other two from

fruiting bodies of fungi, were acetylated. Preparation of the *S. salivarius* (1→3)-α-D-glucan, a bacterial D-glucan, was described in detail in our previous report (Ogawa *et al.*, 1979). The D-glucan acetate (sample A) was prepared by acetylation with trifluoroacetic anhydride (TFAA) (Hamalainen *et al.*, 1957). Starting materials of the fungal (1→3)-α-D-glucans were *Laetiporus sulphureus* (Bull ex Fr.) Murrill supplied by Dr Kreger of the University of Groningen, and *L. sulphureus* (Bull ex Fr.) Bond et Sing which was picked in Kyoto, Japan. The fungal (1→3)-α-D-glucans were extracted from both fungi with 1 M NaOH followed by neutralisation with acetic acid (Jelsma & Kreger, 1979). The former D-glucan was acetylated by TFAA (sample B) and the latter, by acetic anhydride–pyridine (sample C). The degrees of substitution of all acetates (samples A, B and C) were determined with proton NMR spectra to be 3, and the intrinsic viscosities in chloroform solution at 30°C were 1·04 for sample A, 3·24 for B and 1·78 dl g^{-1} for C, respectively, indicating that the molecular weights were in the order B > C > A.

Each sample was dissolved in chloroform and cast into film, from which a well-oriented film could be obtained by stretching in glycerin at 160–180°C. The crystallinity of the triacetate was remarkably improved by annealing the stretched film in water at high temperature, in a sealed bomb, as for the (1→3)-α-D-glucan (Ogawa *et al.*, 1979). Possible deacetylation of the D-glucan triacetate due to annealing was not detected (NMR).

Methods

The density of the D-glucan triacetate film was measured at 25°C by the flotation method in a solution of carbon tetrachloride-*m*-xylene and the X-ray patterns were recorded using a flat-film camera with a Rigaku Geigerflex X-ray diffractometer employing Ni-filtered CuKα radiation generated at 40 kV and 15 mA.

RESULTS

With increasing annealing temperature, the stretched films of all samples (A, B and C) behaved differently from one another.

Sample A prepared from the bacterial (1→3)-α-D-glucan did not show any diffraction spots when the annealing temperature was 120°C

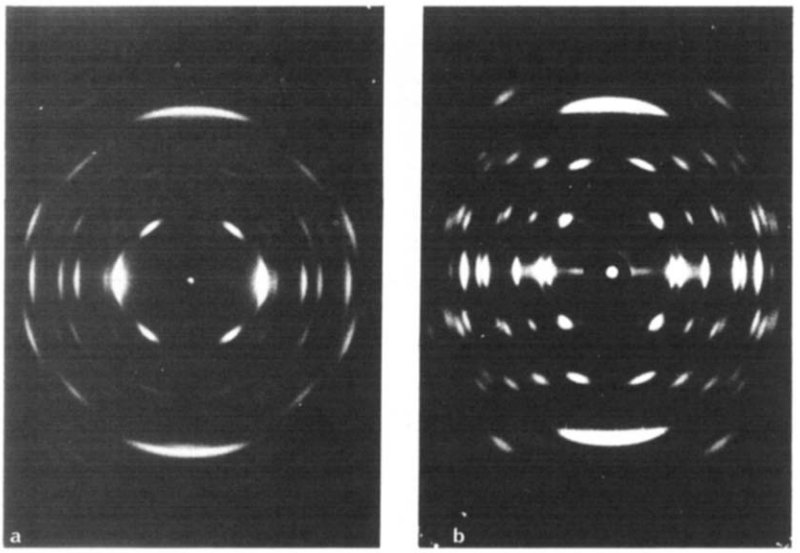

Fig. 1. The fibre X-ray diffraction patterns of (1→3)-α-D-glucan triacetates: (a) GTA I; (b) GTA II. The fibre axis is vertical.

or less. At 140–170°C, it gave a fibre pattern similar to GTA I mentioned below though with less orientation. At 200°C, it lost orientation and showed a powder pattern corresponding to GTA I.

In contrast, sample B obtained from the fungal source exhibited a few diffuse diffraction spots even without annealing, and after annealing at 160–180°C, it gave a clear fibre pattern of GTA I as shown in Fig. 1(a), without any change in polymorphic form due to the annealing. When the annealing temperature was increased above 200°C, sample B showed another fibre pattern similar to that obtained with sample C, GTA II mentioned below, though it lost most of the orientation.

Even without annealing, sample C had many but diffuse spots and with the increase in the annealing temperature up to 215°C, only its crystallinity was improved, i.e. no change in the crystalline form was observed. Figure 1(b) shows the fibre pattern of sample C (GTA II) annealed at 215°C. By annealing above 220°C, sample A lost both orientation and crystallinity, and samples B and C also lost orientation but showed powder patterns corresponding to GTA II.

DISCUSSION

Although an interpretation of the different behaviour of the three samples of (1→3)-α-D-glucan triacetate (A, B and C) is not available at present, it is clear that the D-glucan acetate shows two polymorphic forms, GTA I and GTA II.

All 36 visible diffraction spots in GTA I (Fig. 1(a)), which were observed in samples A and B when annealed in water at low temperatures (140–170°C for A and up to 180°C for B) but were not obtained with sample C, could be indexed with a pseudo-orthorhombic unit cell with $a = 1{\cdot}753$, $b = 3{\cdot}018$ and c (fibre axis) $= 1{\cdot}205$ nm (Tables 1 and 2). The volume of this cell and the density (1·18 g cm^{-3}) observed for the sample B annealed at 180°C are in reasonable agreement with the values calculated for 18 D-glucose triacetate residues per unit cell. Since only the (003) reflection was observed on the meridian, a three-fold

TABLE 1
Crystal Data for the Fibre Patterns of (1→3)-α-D-Glucan Triacetate

Crystalline	*GTA I*	*GTA II*
Crystal system	*Pseudo-orthorhombic*	*Monoclinic*
Lattice parameters		
a (nm)	1·753	1·685
b (nm)	3·018	3·878
c (fibre axis) (nm)	1·205	1·210
γ (degree)		112·2
Density		
ρ (obs.) (g cm^{-3})	1·18	1·34
ρ (calc.) (g cm^{-3})	1·35	1·56
Number of residue	18	24
Number of chain	6	8
Helix parameters		
n	3	3
h (nm)	0·402	0·403

TABLE 2
Observed Spacings and Intensities for the Fibre Pattern of GTA I

h	*k*	*l*	$d_{calc.}$ (nm)	$d_{obs.}$ (nm)	$Int._{obs.}^{a}$	*h*	*k*	*l*	$d_{calc.}$ (nm)	$d_{obs.}$ (nm)	$Int._{obs.}^{a}$
2	0	0	0·877	0·881	vs	2	2	2	0·472	0·469	m
1	3	0	0·873			0	4	2	0·471		
2	2	0	0·758	0·758	m	3	1	2	0·416	0·415	s
0	4	0	0·755			2	4	2	0·415		
3	1	0	0·574	0·571	s	1	5	2	0·414		
2	4	0	0·572			3	3	2	0·387	0·387	w
1	5	0	0·571			0	6	2	0·386		
3	3	0	0·505	0·503	s	2	5	2	0·384		
0	6	0	0·503			4	2	2	0·345	0·346	m
2	5	0	0·497			3	5	2	0·345		
4	2	0	0·421	0·422	s	1	7	2	0·344		
3	5	0	0·420								
1	7	0	0·419			0	0	3	0·402	0·404	s
3	6	0	0·381	0·379	w	1	1	3	0·388	0·388	vs
4	4	0	0·379			0	2	3	0·388		
0	8	0	0·377			2	0	3	0·365	0·366	w
5	1	0	0·348	0·348	m	1	3	3	0·365		
3	7	0	0·347			2	2	3	0·355	0·356	m
2	8	0	0·347			0	4	3	0·355		
5	3	0	0·331	0·330	m	3	0	3	0·331	0·330	s
4	6	0	0·331			3	1	3	0·329		
1	9	0	0·329			2	4	3	0·329		
						1	5	3	0·329		
1	1	1	0·943	0·937	vs	3	3	3	0·315	0·315	w
0	2	1	0·942			0	6	3	0·314		
2	0	1	0·709	0·708	m	4	0	3	0·296	0·296	m
1	3	1	0·707			2	6	3	0·296		
2	2	1	0·642	0·636	m	4	1	3	0·295		
0	4	1	0·640			3	6	3	0·277	0·277	vw
3	1	1	0·518	0·517	s	4	4	3	0·276		
2	4	1	0·517			0	8	3	0·275		
1	5	1	0·516								
3	3	1	0·466	0·466	m	1	1	4	0·296	0·295	m
0	6	1	0·462			0	2	4	0·296		

TABLE 2 *(Continued)*

h	*k*	*l*	$d_{calc.}$ *(nm)*	$d_{obs.}$ *(nm)*	$Int^{a}_{obs.}$	*h*	*k*	*l*	$d_{calc.}$ *(nm)*	$d_{obs.}$ *(nm)*	$Int^{a}_{obs.}$
4	2	1	0·397			2	2	4	0·280	0·280	m
3	5	1	0·397	0·399	s	0	4	4	0·280		
1	7	1	0·396			3	0	4	0·268		
3	6	1	0·364			3	1	4	0·267	0·268	m
4	4	1	0·362	0·362	m	2	4	4	0·267		
0	8	1	0·360			1	5	4	0·267		
5	0	1	0·337	0·336	m	3	3	4	0·259		
5	1	1	0·335			0	6	4	0·259	0·259	vw
5	3	1	0·319			2	5	4	0·258		
4	6	1	0·319	0·318	vw	4	2	4	0·245		
1	9	1	0·318			3	5	4	0·245	0·245	w
						1	7	4	0·245		
1	1	2	0·560	0·557	s						
0	2	2	0·560								
2	0	2	0·497	0·497	m						
1	3	2	0·496								

[a] Abbreviations: vs, very strong; s, strong; m, medium; w, weak; vw, very weak.

screw axis along the *c*-axis is probable. The value of *h*, the advance per residue along the helix axis, 0·402 nm, is close to the virtual bond length of (1→3)-α-D-glucan, 0·422 nm (Ogawa *et al.*, 1981). This indicates that the (1→3)-α-D-glucan triacetate molecule is extended along the fibre axis.

GTA II observed in sample C, and in sample B annealed above 200°C, but not in A, is more complex than GTA I. As shown in Fig. 1(b), among all 54 visible reflections, a large number of reflections from the equatorial to the 6th layer lines could be indexed in terms of axial lengths and the angle of a monoclinic system: $a = 1{\cdot}685$, $b = 3{\cdot}878$, c (fibre axis) $= 1{\cdot}210$ nm and $\gamma = 112{\cdot}2°$. It is notable that the fibre axis has a similar length to that of GTA I.

The observed density (1·34 g cm^{-3}) for sample C annealed at 215°C suggests the cell contains 24 D-glucose triacetate residues (Table 1).

Although a two-fold screw axis along the *c* axis of the unit cell is suggested from the presence of only one meridional reflection, (002), the D-glucan triacetate molecule cannot exist in a two-fold helical conformation because its *h* value (0·605 nm) is larger than the virtual bond length (*ca.* 0·42 nm which is assumed from that for (1→3)-α-D-glucan). A four-fold helix may also be rejected from the conformational analysis for (1→3)-α-D-glucan because of its high conformational energy (Sathyanarayana & Rao, 1972).

From the similarity of the fibre axes of GTA I and GTA II, the D-glucan triacetate molecule in GTA II may also have a three-fold helical conformation (Table 1). In such a case, the D-glucan acetate chain does not coincide with the two-fold screw axis of the unit cell. A number of reflections in GTA II appear above and below the main layer lines: nine spots on the first layer line and one on the second (Fig. 1(b)). The presence of other polymorphic forms, however, is not likely because these spots have various fibre repeat distances as shown in Table 3 and the change in the intensities of all such reflections seemed to be similar to one another with the increase in the annealing temperature:

TABLE 3

Observed Spacings and Intensities for the Fibre Pattern of GTA II

h	*k*	*l*	$d_{calc.}$ (nm)	$d_{obs.}$ (nm)	$Int._{obs.}^{a}$	c^{c} (nm)	*h*	*k*	*l*	$d_{calc.}$ (nm)	$d_{obs.}$ (nm)	$Int._{obs.}^{a}$
1	2	0	1·005	1·027	vs		1	6	2	0·385	0·384	m
0	4	0	0·898	0·904	vs		3	$\bar{6}$	2	0·384		
1	4	0	0·676	0·678	vs		3	2	2	0·364	0·364	vw
3	0	0	0·520	0·519	vs		3	3	2	0·346	0·346	w
3	$\bar{6}$	0	0·496	0·493	s		4	$\bar{3}$	2	0·345		
0	8	0	0·449	0·448	vs		4	$\bar{4}$	2	0·345		
4	$\bar{2}$	0	0·415	0·412	m							
4	$\bar{5}$	0	0·415				0	1	3	0·401	0·398	vs
3	4	0	0·391	0·391	vw		1	0	3	0·391	0·389	vs
4	0	0	0·390				1	$\bar{2}$	3	0·389		
4	$\bar{7}$	0	0·389				0	4	3	0·368	0·369	m
2	8	0	0·338	0·338	s		2	$\bar{4}$	3	0·355	0·356	m
5	$\bar{4}$	0	0·337				1	5	3	0·330	0·331	s
2	10	0	0·288	0·288	w		3	$\bar{6}$	3	0·313	0·312	w
							3	1	3	0·311		

TABLE 3 *(Continued)*

h	*k*	*l*	$d_{calc.}$ (nm)	$d_{obs.}$ (nm)	$Int._{obs.}^{a}$	c^{c} (nm)	*h*	*k*	*l*	$d_{calc.}$ (nm)	$d_{obs.}$ (nm)	$Int._{obs.}^{a}$
0	2	1	1·003	1·069	m		3	$\bar{8}$	3	0·294	0·295	w
1	$\bar{2}$	1	0·939	0·926	vs		3	$\bar{9}$	3	0·283		
1	1	1	0·875	0·866	m		1	8	3	0·282	0·282	vw
1	3	1	0·675	0·674	s		3	4	3	0·281		
2	$\bar{3}$	1	0·672									
———			——	0·619[b]	w	1·4	1	2	4	0·290	0·290	w
———			——	0·585[b]	vw	1·3	2	0	4	0·282		
———			——	0·546[b]	s	1·15	1	$\bar{5}$	4	0·282	0·281	m
1	5	1	0·519	0·522	m		2	$\bar{4}$	4	0·280		
2	$\bar{7}$	1	0·486	0·484	w		0	6	4	0·270		
3	$\bar{5}$	1	0·483				2	$\bar{6}$	4	0·270	0·269	w
———			——	0·431[b]	s	1·4	1	5	4	0·268		
3	$\bar{8}$	1	0·405	0·408	s		3	2	4	0·252		
———			——	0·399[b]	m	1·5	2	5	4	0·251	0·251	vw
———			——	0·390[b]	m	1·5	0	8	4	0·251		
———			——	0·366[b]	w	1·3						
4	1	1	0·356	0·354	m		2	3	5	0·222	0·221	vw
4	$\bar{8}$	1	0·356					|		|		
2	7	1	0·353				3	0	5	0·220		
3	$\bar{10}$	1	0·352				3	$\bar{11}$	5	0·197	0·197	vw
———			——	0·341[b]	w	1·25		|		|		
1	$\bar{12}$	1	0·307	0·306	vw		5	$\bar{6}$	5	0·196		
4	4	1	0·306				3	$\bar{13}$	5	0·187	0·186	vw
3	$\bar{12}$	1	0·306					|		|		
4	$\bar{11}$	1	0·306				5	$\bar{11}$	5	0·185		
———			——	0·288[b]	vw	1·45						
							0	6	6	0·191	0·190	vw
0	0	2	0·605	0·605	s			|		|		
0	2	2	0·573	0·573	vs		2	$\bar{7}$	6	0·189		
1	$\bar{1}$	2	0·569				2	5	6	0·184	0·183	vw
0	4	2	0·502	0·501	s			|		|		
2	0	2	0·478	0·480	vw		4	$\bar{4}$	6	0·182		
2	2	2	0·438	0·436	s							
———			——	0·409[b]	m	1·3						

[a] Abbreviations: vs, very strong; s, strong; m, medium; w, weak; vw, very weak.
[b] Split reflections.
[c] The fibre axis length for each split reflection.

it is difficult to consider that the crystallinity of two or more polymorphic forms is improved simultaneously to the same extent. Thus, the occurrence of these split reflections may be due to a chain disorder, e.g. the presence of a non-integral helical chain in the crystal.

Effect of acetylation on the molecular conformation of (1→3)-α-D-glucan

In the four polymorphic forms for (1→3)-α-D-glucan which have been reported, all of the D-glucan molecules are fully extended two-fold helices and they have almost the same *h* value (Jelsma & Kreger, 1980; Ogawa *et al.*, 1980). In the present case, we propose that the acetyl derivatives of the D-glucan are in an extended three-fold helical conformation in GTA I and basically the same in GTA II.

When the stretched D-glucan triacetate films of all samples (A, B and C) were deacetylated in sodium methylate–methyl alcohol solution, the films were somewhat elongated along the stretching axis and showed fibre patterns similar to the regenerated form (polymorph IV) of the (1→3)-α-D-glucan (Ogawa *et al.*, 1979). This indicates that, by deacetylation, the conformation of the D-glucan backbone chain changes from the extended three-fold helix ($h = 0{\cdot}402$ nm) to the fully extended two-fold helix ($h = 0{\cdot}422$ nm) with a little elongation; that is, the D-glucan molecule is shrunk after acetylation. The inter-residue hydrogen bonds of the (1→3)-α-D-glucan molecule which stabilise the fully extended two-fold helix (Ogawa *et al.*, 1981) are eliminated by the acetylation and as a result, the backbone chain is shrunk.

ACKNOWLEDGEMENTS

We are grateful to Dr D. R. Kreger of the University of Groningen for the gift of *L. sulphureus* (Bull ex Fr.) Murill, to Professor A. Misaki of Osaka City University for supplying a sample of the bacterial (1→3)-α-D-glucan, and to Professor A. Sarko of State University of New York, College of Environmental Science and Forestry, for helpful suggestions on analysing the X-ray fibre patterns.

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