X-ray Study of Chitosan L- and D-Ascorbates

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Well-defined X-ray fiber patterns of L- and D-ascorbates of chitosan were obtained by immersing a tendon chitosan, prepared from a crab tendon chitin by a solid-state *N*-deacetylation, in respective ascorbic acid water−isopropyl alcohol solutions, and heating them at 70 °C. The crystalline unit cell of the L-ascorbate of chitosan was a monoclinic (pseudoorthorhombic) with the following dimensions: $a = 1.122$; $b = 1.177$; c (fiber axis) = 1.040 nm; and $\beta = 90°$. The presence of a $P2_1$ space group with b axis unique was suggested. The visible reflections on the fiber diagram of the chitosan D-ascorbate could be indexed in terms of a monoclinic unit cell with $a = 0.962$, $b = 1.349$, c (fiber axis) = 1.030 nm, and $\gamma = 96.7°$; no space group could be assigned, however. Although no 2_1 symmetry is present along the fiber axes in these crystals, the similarity of both fiber axes to that of the unreacted chitosan (1.043 nm) suggested that the extended 2-fold helical conformation of chitosan is retained in the backbone chitosan chain of each ascorbate. The observed density values of both ascorbates suggested that two chitosan chains, but no water molecule are present in each unit cell of L- and D-ascorbates. It was suggested during the preparation of respective L- and D-ascorbate of chitosan that D-ascorbic acid has a higher reactivity with chitosan to make the salt than the L isomer has.

Introduction

Polysaccharides have been promising biodegradable polymer materials and under intensive investigation for their industrial purposes. Chitosan, a polymer of β-(1→4)-linked 2-amino-2-deoxy-D-glucose, is one of the most topical polysaccharides so far. Crystal structures of chitosan have been studied since the work of Clark and Smith[1] in 1937. These studies have revealed that chitosan molecule takes up two kinds of conformation in the crystals: an extended 2-fold[1−6] and a right-handed 8-fold helical structure,[7] although final analysis of the crystal and molecular structure of chitosan has been done for its anhydrous polymorph only.[5,6]

Since chitosan has a regular distribution of aliphatic primary amino groups on the chain, it gives salts when it reacts with an inorganic or organic acids. X-ray fiber diffraction study of several crystallized inorganic acid salts of chitosan has suggested that chitosan acid salts take up two different conformations.[8] One, called type I salt, retained the 2-fold helix of unreacted chitosan although they were different crystals to one another. The other, called type II salt, has an extended 8-fold helical conformation. Crystals of type I salts are anhydrous forms, whereas those of type II are hydrated. Salts formed with HNO_3, HBr, and HI take up the former structure, and those with HF, HCl, and H_2SO_4, the latter. Despite differing anion sizes, all the type II salts gave fiber patterns very similar to one another; that is, they have identical unit-cell dimensions, suggesting that these anions are not present regularly in the crystals and that the fiber patterns are of the backbone chitosan chains only. The chirality of the 8-fold helix is the right handed since their fiber patterns were very similar to that obtained by Cairns et al.[7] A solid-state ^{13}C NMR study of these crystalline salts[9] revealed that the two types of helical structures were easily distinguishable by their spectra. In addition, other acid salts of chitosan including organic acid salts, $HClO_4$, HIO_4, H_3PO_4, HCOOH, and CF_3COOH were also classified in the two types of conformation by the NMR study.

Chitosan and partially *N*-acetylated chitosan are easily derived by deacetylation of chitin, poly(*N*-acetyl-D-glucosamine). They are expected to be applied in medical fields such as drug carriers in DDS.[10−12] Chitosan makes a salt when reacts with L-ascorbic acid, vitamin C, suggesting that chitosan may act as a carrier

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Table 1. Preparation of Tendon Chitosan Ascorbates[a]

concn of ascorbic acid (M)	reaction temp (°C)	reaction time (h)	fiber pattern
	L-Ascorbic Acid		
4	room	1.5	chitosan
2	40	1.5	chitosan
2	70	3.0	a mixture of the salt and chitosan
4	70	3.0	the salt
4	75	1.0	the salt
	D-Ascorbic Acid		
2	room	3.0	a mixture of the salt and chitosan
2	70	3.0	the salt

[a] A tendon chitosan was immersed in a mixture of an aqueous ascorbic acid and isopropyl alcohol (1:3 v/v) under nitrogen atmosphere.

of ascorbic acid. This stimulated us to study the chain conformation of the ascorbic acid salt of chitosan. In addition, we are interested in the comparison of chain conformation of the salt with that of the salt of optical isomer, D-ascorbic acid, which is not present in nature but is widely utilized in food industries as an antioxidant because it has no toxicity to human being and is cheaper than the L-isomer.

Experimental Section

Materials and Methods. Tendon chitosan was prepared from the chitin of a crab tendon. *Chionecetes opirio* O. Fabricus, by a complete *N*-deacetylation described in the previous paper.[13] Both L- and D-ascorbic acids were of reagent grade. In order to prevent the decomposition of L- or D-ascorbic acid when dissolved in water, all the preparation of chitosan ascorbates was done under nitrogen atmosphere. Dehydroascorbic acid was prepared from L-ascorbic acid, according to the methods of Ohmori and Takagi.[14]

The density of each chitosan ascorbate was measured by a flotation method in carbon tetrachloride−ethylene dibromide at 25 °C. The X-ray fiber diffraction patterns were recorded by using a flat-film camera at 100% relative humidity in a helium atmosphere or under vacuum with a Rigaku Geriger-flex X-ray diffractometer employing Ni-filtered Cu Kα radiation generated at 40 kV and 15 mA.

Results and Discussion

Since chitosan dissolves with an aqueous ascorbic acid solution, chitosan-L-ascorbic acid salts were prepared by immersing the tendon chitosan in a mixture of 4 M ascorbic acid aqueous solution and isopropyl alcohol (1:3, v/v) under nitrogen atmosphere at room temperature for 1.5 h, followed by washing with 75% aqueous isopropyl alcohol and isopropyl alcohol and dried in air. The resultant sample, however, showed an X-ray fiber pattern of hydrated chitosan polymorph, the so-called tendon chitosan polymorph,[15] indicating that the salt formation was not enough. As shown in Table 1, a higher concentration of ascorbic acid and higher temperature were required in order to get a fiber pattern of the chitosan-L-ascorbate. The decomposition of the ascorbic acid which may be expected to occur during the heating even though under nitrogen atmosphere could be ruled out since the similar treatments for the chitosan in a mixture of aqueous dehydroascorbic acid and isopropyl alcohol (1:3, v/v) gave the chitosan's tendon polymorph only, indicating no reaction between chitosan and dehydroascorbic acid.

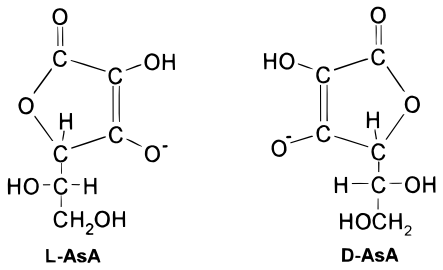

Figure 1. Chemical structures of L-ascorbic (left) and D-ascorbic acids (right) in monoanion forms.

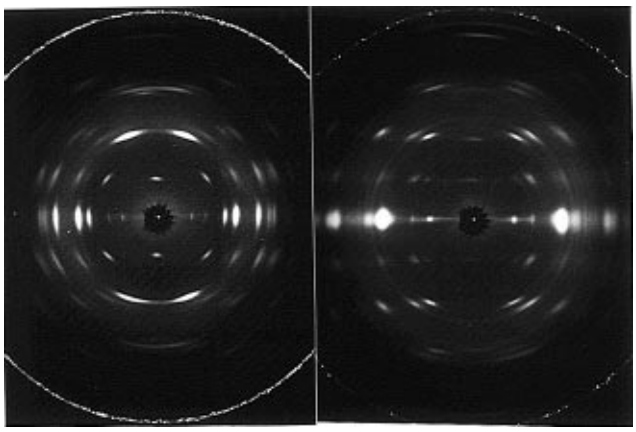

Figure 2. Fiber diffraction patterns of L-ascorbate (a, left) and D-ascorbate (b, right) of chitosan.

The D-ascorbic acid salt of chitosan was prepared by the similar method with the L-ascorbic acid salt, but lower concentration of the acid and lower temperature than the preparation for the L-ascorbate of chitosan was required (Table 1) suggesting that D-ascorbic acid may have a higher reactivity with chitosan to make the salt than L-ascorbic acid has.

Ascorbic acid is known to have two pK values: pK_1 4.25[16] and pK_2 11.79.[17] In the present experimental condition, the dissociation of ascorbic acid is considered to occur at pK_1, that is, only the hydroxyl group at C3 is dissociated (Figure 1). And the monoanion reacts with the amino group of chitosan chain to make the salt.

Figure 2a shows a fiber pattern of chitosan L-ascorbate. All 34 reflections observed on the 0th-, 1st-, 2nd-, 3rd-, and 4th-layer lines could be indexed in terms of a monoclinic (pseudoorthorhombic) unit cell with the following dimensions: $a = 1.122$; $b = 1.177$; c (fiber axis) $= 1.040$ nm; $\beta = 90°$ (Tables 2 and 3). The absence of (0 odd 0) reflection on the equatorial layer line suggested that the probable space group is $P2_1$ with b axis unique. The observed density was in good agreement with the density calculated for 4 glucosamine ascorbate residues/unit cell. The absence of water molecules in the cell was supported by there being no observed change in the fiber pattern when the chitosan L-ascorbate was X-rayed at 100% relative humidity or under vacuum. The presence of odd meridional reflections, (0 0 1) and (0 0 3), indicated no 2_1 screw axis along the c axis (Table 3). However, the similarity of the present c-axis length

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Table 2. Crystal Data for the Tendon Chitosan Ascorbates

	L-ascorbate	D-ascorbate
crystal system	monoclinic (pseudoorthorhombic)	monoclinic
space group	$P2_1$ (2nd setting)	
lattice parameters		
a (nm)	1.122 (0.005)	0.962 (0.005)
b (nm)	1.177 (0.006)	1.349 (0.004)
c (fiber axis) (nm)	1.040 (0.003)	1.030 (0.002)
β (deg)	90	
r (deg)		96.7 (0.2)
vol of the unit cell (nm^3)	1.373	1.327
density		
ρ (obsd) ($g\ cm^{-3}$)	1.59	1.63
ρ (calcd) ($g\ cm^{-3}$)	1.63	1.69
unit-cell composition		
no. of glucosamine ascorbate residues (repeating unit)	4	4
no. of water molecules	0	0
no. of chains	2	2

Table 3. Observed Spacing and Intensity for the Tendon Chitosan L-Ascorbate

			spacing, nm		intensity				spacing, nm		intensity
h	k	l	calcd	obsd	obsd[a]	h	k	l	calcd	obsd	obsd[a]
1	0	0	1.122	1.132	W	1	2	2	0.368	0.369	M
1	1	0	0.812	0.828	W	2	1	2	0.363		
0	2	0	0.589	0.593	S	2	2	2	0.320	0.311	VW
1	2	0	0.521	0.521	VS	0	3	2	0.313		
2	2	0	0.406	0.407	S	3	0	2	0.304	0.303	M
3	0	0	0.374	0.378	M	1	3	2	0.302		
1	3	0	0.370			3	1	2	0.294	0.293	W
3	1	0	0.356	0.355	M	2	3	2	0.274	0.274	VW
2	3	0	0.322	0.323	W	3	2	2	0.270		
3	2	0	0.316			0	0	3	0.347	0.347	(M)[b]
4	1	0	0.273	0.271	M	0	1	3	0.333	0.325	M
3	3	0	0.271			1	0	3	0.331	0.322	M
0	0	1	1.041	1.034	(M)[b]	1	1	3	0.319		
1	1	1	0.640	0.637	M	0	2	3	0.299	0.299	VW
0	2	1	0.512	0.517	VW	2	0	3	0.295		
1	2	1	0.466	0.467	W	1	2	3	0.289	0.290	M
2	1	1	0.455			2	1	3	0.286		
0	3	1	0.367	0.370	M	2	2	3	0.264	0.267	W
3	0	1	0.352	0.346	M	0	3	3	0.260	0.255	VW
1	3	1	0.349			3	0	3	0.254		
3	1	1	0.337	0.336	W	1	3	3	0.253		
2	3	1	0.307	0.308	W	3	1	3	0.249		
3	2	1	0.302			0	0	4	0.260	0.253	(S)[b]
0	1	2	0.476	0.476	VS	0	1	4	0.254		
1	0	2	0.472			1	0	4	0.253		
1	1	2	0.438	0.437	W	1	1	4	0.248		
				0.389	W[c]	0	2	4	0.240		

[a] Abbreviations: VS, very strong; S, strong; M, medium; W, weak; VW, very weak. [b] ( ): meridional reflections. [c] Reflection displaced from the secnd layer line.

(1.040 nm) with that for free chitosan (1.043 nm)[5] suggested that the backbone chitosan chain of the salt molecule still retained the extended 2-fold helical conformation. This and the presence of $P2_1$ space group with the second setting indicate that two chains of the chitosan L-ascorbate are packed in an antiparallel fashion to each other in the unit cell (Table 2). The innermost reflection of the upper third-layer line is displaced from the meridian (Figure 2a). When tilting the sample, there was observed a new reflection having similar d spacing to that of the displaced reflection. This fact may suggest that there is included a small amount of inclined chitosan fiber in the tendon chitosan sample. Since it was prepared from the natural tendon chitin by the solid-state deacetylation in order to prevent a loss of the orientation, it was impossible to prepare a sample having no such displaced reflection. The similar situation occurred in the third reflection on the second layer line (Figure 2a and Table 2) which was not joined for the determination of the unit-cell parameters of the present crystal.

Table 4. Observed Spacing and Intensity for the Tendon Chitosan D-Ascorbate

			spacing, nm		intensity				spacing, nm		intensity
h	k	l	calcd	obsd	obsd[a]	h	k	l	calcd	obsd	obsd[a]
1	0	0	0.956	0.935	S	0	0	2	0.515	0.515	(S)[b]
0	2	0	0.670	0.667	M	1	0	2	0.453	0.450	S
1	2	0	0.521	0.521	M	1	−1	2	0.437		
2	0	0	0.478	0.470	VS	1	1	2	0.422	0.416	S
2	−1	0	0.468			0	2	2	0.408		
2	1	0	0.434	0.427	M	2	0	2	0.350	0.351	M
1	−3	0	0.424			2	−1	2	0.346		
2	−2	0	0.412	0.412	M	0	4	2	0.281	0.275	M
1	3	0	0.388	0.388	W	1	−4	2	0.277		
2	2	0	0.369	0.369	W	3	0	2	0.271		
2	−3	0	0.347	0.352	W	3	−1	2	0.271		
0	4	0	0.335	0.328	W	0	1	3	0.333	0.333	W
1	−4	0	0.328			1	0	3	0.323	0.325	M
3	0	0	0.319	0.310	S	1	−1	3	0.317		
3	−1	0	0.318			1	1	3	0.311	0.309	M
2	3	0	0.309			0	2	3	0.306		
2	−4	0	0.291	0.291	S	2	0	3	0.279	0.276	VW
0	0	1	1.030	1.023	(S)[b]	2	−1	3	0.277		
1	0	1	0.701	0.683	S	0	3	3	0.272		
1	1	1	0.600	0.603	S	2	1	3	0.269	0.262	VW
0	2	1	0.562	0.575	S	1	−3	3	0.267		
1	2	1	0.465	0.452	M	2	−2	3	0.264		
2	0	1	0.434	0.421	M	1	3	3	0.257		
2	−1	1	0.426			0	0	4	0.258	0.255	(M)[b]
0	3	1	0.410			1	0	4	0.249	0.243	S
2	1	1	0.400			1	−1	4	0.246		
0	4	1	0.319	0.313	VW	1	1	4	0.243		
1	−4	1	0.313			0	2	4	0.240		
3	0	1	0.304	0.296	VW	1	−2	4	0.236		
3	−1	1	0.304								
2	3	1	0.296								
1	4	1	0.293								
2	−4	1	0.280	0.278	VW						

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

As shown in Figure 2b the D-ascorbate of chitosan gave different fiber pattern with the L-ascorbate. The 33 visible reflections from the equator to the 4th-layer lines led to a monoclinic unit cell: $a = 0.962$; $b = 1.349$; c (fiber axis) = 1.030 nm; $\gamma = 96.7°$ (Tables 2 and 4). The absence of any water molecule in the cell was also confirmed not only by the observed density value but also from the fact that no change in the fiber pattern was observed when the chitosan D-ascorbate was irradiated at 100% relative humidity or under vacuum. Unfortunately, any space group could not be considered because of the presence of both odd and even meridional reflections: (0 0 1), (0 0 2), and (0 0 4). However, like the story of chitosan L-ascorbate, the backbone chitosan chain of the salt retained the extended 2-fold helix because of the similarity of the fiber axis length (10.30 nm) with that for the free chitosan, and two chains of the salt were also packed in the unit cell (Table 2).

In the present L- and D-ascorbates the extended 2-fold helical conformation of the unreacted chitosan chain was retained and both crystals were anhydrous, indicating that they are classified into type I salt of the chitosan acid salts.[8] The difference of reactivity between L- and D-ascorbic acid with chitosan may be applied to the optical resolution of ascorbic acid.

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