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Two different molecular conformations found in chitosan type II salts

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Abstract

The type II structure of chitosan acidic salts prepared from crab tendon in solid state was studied using an X-ray fiber diffraction technique together with the linked-atom least-squares (LALS) technique. The cylindrical Patterson method was applied to confirm the molecular conformation of the chitosan. It was shown that there are two different helical conformations for type II salts. One is the relaxed twofold helix having a tetrasaccharide as an asymmetric unit as found in chitosan·HCl salt, which was previously reported as a conformation of chitosan·HCOOH salt. The other is the fourfold helix having a disaccharide as an asymmetric unit newly found in chitosan·HI salt.

Keywords: Chitosan acidic salt; Chitosan conformation; Cylindrical Patterson method

1. Introduction

Chitosan, poly [(1→4)-β-D-glucosamine], is the N-deacetylated derivative of chitin that is widely distributed as a structural component of crustaceans. This biopolymer is a versatile material due to its properties such as biodegradability,[1] biocompatibility,[2] wound-healing accelerator,[3,4] flocculant[5,6] and chelation agent.[7] The structure of chitosan has been studied in both the solid and liquid states in order to get a better understanding and develop those properties.

Chitosan derived from chitin was shown to exhibit a hydrated crystalline form which can be converted to an anhydrous crystalline form by annealing in water at 240 °C.[8] Structural studies by X-ray diffraction showed that both hydrated and anhydrous forms of chitosan adopt a twofold helical conformation having monosaccharide as an asymmetric unit with about 10 Å fiber period,[9,10] a structure that is similar to other β-(1→4)-linked polysaccharides such as chitin and cellulose. The conformation of chitosan acid salts, however, can be classified into two types, type I and II salts, according to their fiber periods of about 10 and 40 Å, respectively. The extended twofold conformation of the starting chitosan was retained in type I salts, while the conformation of the type II salts was changed from the original extended one to a somewhat relaxed one as the fiber period lengthens to about 40 Å. It is interesting that only chitosan among β-(1→4)-linked polysaccharides was found to have such a long fiber period. Furthermore, some type II salts were found to transform to anhydrous chitosan when maintained 100% RH or when immersed in a solution of 2-propyl alcohol.[11] The explanation of such behaviors is expected to be obtained if the detailed structure of type II salts is clarified.

The conformation of type II was once proposed as a left-handed eightfold helix (8/5).[12] However, an alternative relaxed 2/1-helical conformation having a tetrasaccharide as an asymmetric unit was found to be preferable.[13,14] Generally, the diffraction patterns of type II salts look similar to each other. Therefore, it was thought that the chitosan chains in various type II

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salts took the same conformation. In this study, however, the diffraction pattern of the chitosan·HI salt showed some different features from those of the relaxed 2/1-helical structure.

2. Results and discussion

Although the diffraction patterns of chitosan·HI and chitosan·HCl salts were very similar, there are some variations (Fig. 1). Noticeable differences on the meridian and equator are shown in Fig. 2. The meridional reflections on the 8th, 12th and 16th layer lines could be observed in the patterns of the chitosan·HCl salt, whereas the additional strong reflection on the 4th layer line was found in chitosan·HI salt. The *110* reflection on the equator is very strong in the chitosan·HCl pattern salt, whereas this reflection could not be observed in the pattern of the chitosan·HI salt. Two spots on the 1st layer line ($d = 3.77$ and 3.62 Å) could be observed in the chitosan·HCl salt, but only one intense spot ($d = 3.81$ Å) could be observed on the equator in the pattern of chitosan·HI salt.

A total of 56 and 58 diffraction spots up to 18 layer lines could be observed in the diffraction patterns of chitosan·HCl and chitosan·HI salts, respectively (Fig.

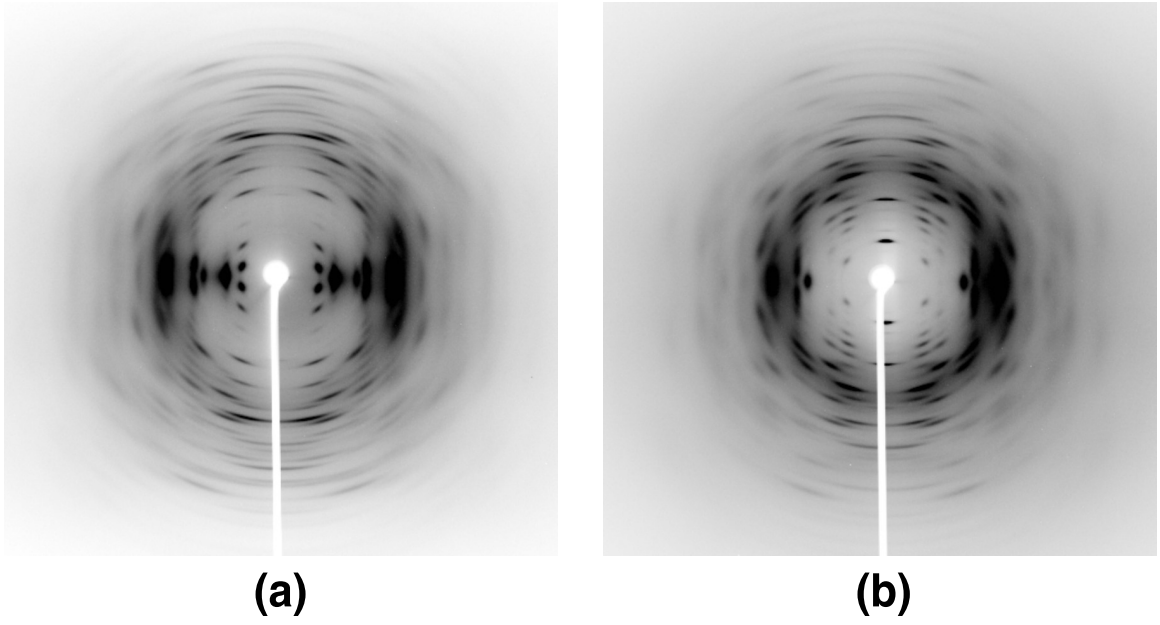

Fig. 1. Synchrotron X-ray diffraction patterns of: (a) chitosan·HCl; and (b) chitosan·HI salts recorded on an imaging plate (RAXIS IV^{++}, Rigaku) at BL40B2 in SPring-8, Hyogo, Japan.

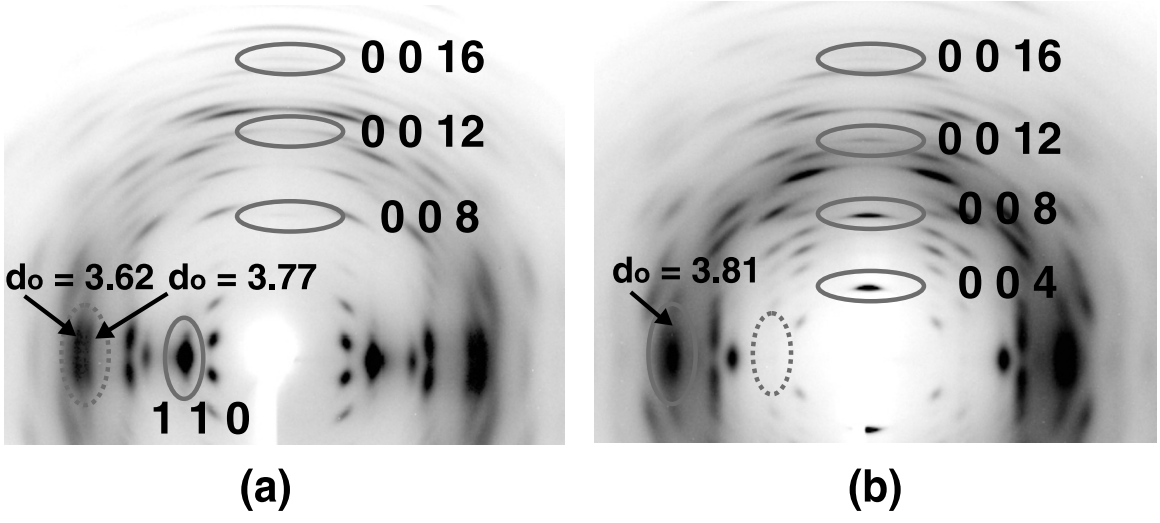

Fig. 2. Noticeable differences on meridian and equator between chitosan type II salts: (a) chitosan·HCl; and (b) chitosan·HI salts.

Table 1
Crystal data of chitosan type II salts

	Chitosan·HCl	Chitosan·HI
Crystal system	monoclinic	tetragonal
Space group	$P2_1$	$P4_1$
Unit cell dimensions		
a (Å)	10.67(3)	10.78(2)
b (Å)	11.41(3)	10.78(2)
c (fiber axis, Å)	40.31(7)	40.54(8)
V (Å^3)	4908	4711
Density		
D_{obs} (g cm^{-3})	1.40	1.81
D_{calc} (g cm^{-3})	1.40	1.81
Observed spots	56	58
Meridional reflections	*0 0 8, 0 0 12, 0 0 16*	*0 0 4, 0 0 8, 0 0 12, 0 0 16*
Chitosan chains running through the unit cell	2	2
Glucosamine residues	16	16
Halide ions in unit cell	16	16
Water molecules [a]	56	28

[a] The number of water molecules shown above was calculated based on the observed densities.

1). A rectangular unit cell with dimensions of $a = 10.67$, $b = 11.41$, c (fiber axis) $= 40.31$ Å and $\gamma = 90°$ was obtained for the chitosan·HCl salt. On the other hand, a tetragonal crystal system with dimensions of $a = b = 10.78$, c (fiber axis) $= 40.54$ Å was obtained for chitosan·HI salt. The unit cell volumes of these two salts are quite similar. The crystal data are summarized in Table 1.

From the observed densities and unit cell volumes (Table 1), the number of polymer chains running through the unit cell is two in both cases. Each chain has an octasaccharide in the c-repeat unit. Since each amino group of chitosan interacts with an acid molecule to make the salt complex, there should be 16 halide ions in the unit cell. In order to get good agreement between the observed and calculated densities, a total of 56 and 28 water molecules should be included in the unit cells of chitosan·HCl and chitosan·HI salts, respectively. The difference in the number of water molecules is consistent with weight losses of chitosan·HCl (16.8%) and chitosan·HI salts (9.3%) obtained by thermogravimetric measurement. This difference in water molecules can be understood by considering the volume of halide anion. That is, since the volume of an iodide ion is 1.9 times bigger than that of a chloride ion, the unit cell of the chitosan·HI salt could accommodate only half the number of water molecules of the chitosan·HCl salt.

The structure of chitosan·HCl salt was once reported as either left- or right-handed helices that have eight residues in three turns.[15] Later, the left-handed 8/3 helix (8/5) was reported to be more preferable.[12] Nevertheless, this conformation was inconsistent with the observed *0012* reflection and also with the significant splitting of the C-1, C-3 and C-6 ^{13}C NMR signals.[16] The systematic absence of the meridional reflections suggested both two- and fourfold conformations of this polymer. The structural study based on the linked-atom least-squares (LALS) method revealed that the fourfold helix is not preferable because of its poor agreement between observed and calculated structure amplitudes. On the other hand, the agreement was much better in the case of the relaxed 2/1-helical conformation. The most plausible molecular structure obtained so far is shown in Fig. 3(a), which is very similar to the one reported for chitosan·HCOOH.[13]

Judging from the number of saccharide residues in the unit cell of the chitosan·HI salt (Table 1), it is impossible to have a crystallographic fourfold symmetry between chitosan chains. Therefore, the molecular conformation must have a fourfold helical symmetry with a disaccharide in the asymmetric unit. This conformation is also supported by the strong meridional (*004*)

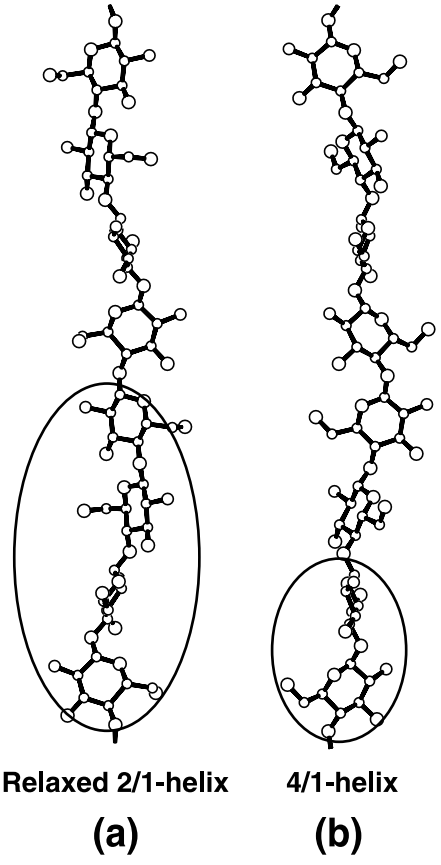

Fig. 3. Molecular conformations of: (a) chitosan·HCl; and (b) chitosan·HI salts. Ellipsoids represent helical asymmetric unit.

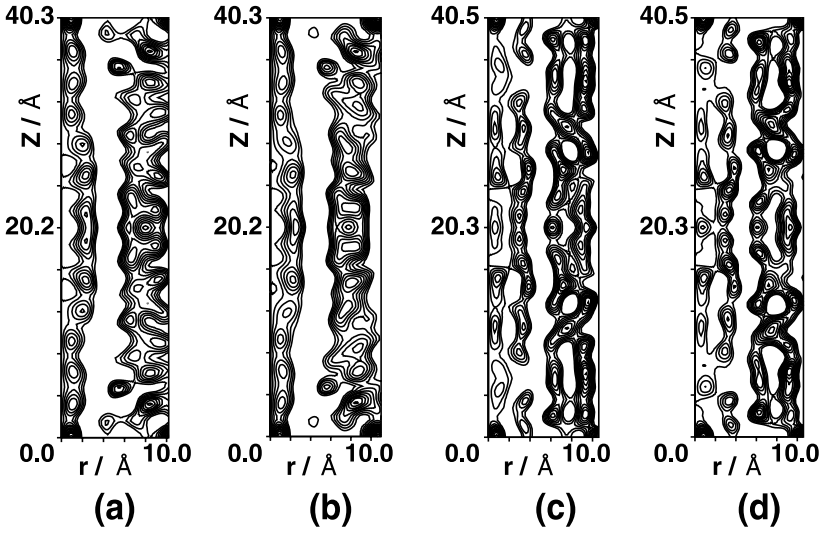

Fig. 4. Cylindrical Patterson maps of chitosan type II salts synthesized by using: (a) the observed intensities of chitosan·HCl salt; (b) the calculated intensities of chitosan·HCl salt shown in Fig. 3(a); (c) the observed intensities of chitosan·HI salt; (d) the calculated intensities of chitosan·HI salt shown in Fig. 3(b).

reflection in the diffraction pattern. Although both right- and left-handed helices are possible, the relaxed potential energy surfaces of chitobiose[17] based on the MM3 force field suggested that the right-handed helix (4/1), rather than the left-handed helix (4/3), should be a plausible conformation of this salt. The best molecular structure with the 4/1-helical symmetry obtained so far is shown in Fig. 3(b). A detailed discussion about molecular conformation and structural analysis of this polymer will be given elsewhere.

In order to confirm the relaxed 2/1-helical conformation in the chitosan·HCl salt, together with the 4/1-helical conformation in the chitosan·HI salt, cylindrical Patterson maps were synthesized by using observed and calculated intensities in both cases (Fig. 4). Calculated intensities were obtained from the crystal structures with the corresponding molecular conformations shown in Fig. 3. In all four cases, the Patterson map consists of two columns along the z-axis at about $r = 0$ and 8 Å, which correspond to the intra- and inter-molecular vector peaks, respectively. The locations of the former vector peaks are closely related to the molecular conformation. Since these locations in the map obtained from the observed intensities of the chitosan·HCl salt are very different from those of the chitosan·HI salt, it is clear that these two salts have a different molecular conformation. On the other hand, the resemblance of the two maps obtained from the observed and calculated intensities in both chitosan·HCl (Fig. 4(a and b)) and chitosan·HI (Fig. 4(c and d)) cases is remarkable. These facts clearly confirm that chitosan molecules take a different conformation in these two salts. As shown in the above, these are the relaxed 2/1-helical conformation for the chitosan·HCl salt and the newly found 4/1-helical conformation for chitosan·HI salt.

3. Conclusion

The molecular conformation of chitosan type II salts were determined by the X-ray fiber diffraction method, the LALS refinement technique, together with the cylindrical Patterson method. Two different conformations could be found in type II salts as confirmed by the agreement of cylindrical Patterson map. One is the relaxed 2/1-helical conformation that exploits a tetrasaccharide as an asymmetric unit in chitosan·HCl salt and the other is the newly found 4/1-helical conformation having a disaccharide as an asymmetric unit in the chitosan·HI salt.

4. Experimental

The starting material, tendon chitosan, was prepared from the tendon of *Chionoecetes opilio* O. Fabricius crab. Inorganic compounds were first removed by immersing the specimen in 2 M HCl overnight. Then the specimen was deacetylated twice with 50% aq NaOH at 110 °C for 2.5 h under a nitrogen atmosphere. The degree of N-acetylation by the colloidal titration and the viscosity-average degree of polymerization of the tendon chitosan were almost 0% and at least 10,800, respectively.[18] Chitosan·HCl salt was prepared by immersing chitosan in a solution of 0.4 M HCl and 2-propanol (1:3) at room temperature (rt) for 2 h. Chitosan·HI salt was obtained by immersing chitosan in 6 M hydriodic acid (HI) for 24 h at 4 °C. The complexes were then washed with 75% 2-propanol, followed by pure 2-propanol. The specimens were dried in air before the X-ray measurements were taken.

X-ray diffraction patterns of chitosan type II salts were recorded on an imaging plate (RAXIS IV^{++}, Rigaku) using synchrotron radiation ($\lambda = 1.00$ Å) at BL40B2 in the SPring-8, Hyogo, Japan. The typical camera length and exposure time were 150 mm and 3 min, respectively. The camera length was calibrated by the characteristic *d*-spacing of silicon (3.1355, 1.9201, 1.6375, 1.3577 and 1.2459 Å). The in-house data processing software was used to obtain the X-ray intensity (I_0) for each diffraction spot and to determine the unit cell parameters.[9,19,20] The measured intensities (I_0) were corrected for the Lorentz and polarization factors. The absorption effect was not corrected in this study.

The specimens were examined by thermogravimetry (Thermo Plus TG8120, Rigaku) at a scanning rate 10 °C min^{-1} from rt to 300 °C.

Densities of the specimens were measured by a floatation method with a solution of 1,2-dibromoethane and 1,2-dichloroethane.

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