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Carbohydrate Polymers 41 (2000) 237–247

Carbohydrate Polymers

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Structural diversity of chitosan and its complexes

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Accepted 20 September 1999

Abstract

The lattice parameters for a number of chitosan specimens were classified into two, hydrated and anhydrous, groups. Polymers in them have the same extended two-fold helical structure, while the packing arrangements and water contents are quite different. Chitosan complexes with acid or metal salts can be classified into two types. Type I has a 10.3 Å axial repeat similar to hydrated and anhydrous chitosan, while Type II has a 40 Å axial repeat; by using X-ray data from highly oriented specimens, plausible crystal structures have been obtained in each case. The Type II conformation corresponds to a less-extended two-fold helical model with a tetrasaccharide repeat in a helical asymmetric unit.

Keywords: Chitosan structure; Chitosan complex with acid or metal salt; Fiber diffraction

1. Introduction

Chitin is the prominent structural polysaccharide in the exoskeleton of insects, crustaceans and invertebrates in general. The shells of crabs and lobsters are common sources of chitin. In many respects, chitin plays an analogous role to collagen in the higher animals and cellulose in terrestrial plants. It is assumed that chitin is even more widespread and abundant in nature than cellulose (Rees, 1977). However, its utilization is very difficult because of its insolubility in water and many commercial solvents. On the other hand, chitosan, the *N*-deacetylated chitin (Fig. 1), is soluble in various acidic solvents such as dilute hydrochloric, formic and acetic acids and also has a remarkable ability to form specific complexes with a number of ions including transition and post-transition metal ions (Ogawa, Oka & Yui, 1993). Therefore, chitosan has received much attention as a functional biopolymer for diverse applications due to the above abilities. These functions undoubtedly depend not only on the chemical structure but also the molecular conformation of chitosan together with its packing arrangement. Therefore, structural studies of these compounds become important for better understanding of their functions, for their utilization and also for improvement of their functions.

Many different X-ray diffraction patterns from chitosan, chitosan acid salts and chitosan complexes with metal salts have been reported so far. The X-ray fiber diffraction data of chitosan was first derived from the solid-state deacetylated product of a lobster tendon chitin (Clark & Smith, 1937). The tendon chitosan shows a hydrated crystalline form which can be converted to an anhydrous form by annealing at about 240°C in water (Ogawa, Hirano, Miyanishi, Yui & Watanabe, 1984). Both the crystal structures of chitosan hydrated and anhydrous form were analyzed precisely (Okuyama, Noguchi, Miyazawa, Yui & Ogawa, 1997; Okuyama, Noguchi, Hanafusa, Osawa & Ogawa, 1999a; Yui, Imada, Okuyama, Obata, Suzuki & Ogawa, 1994).

By immersing hydrated chitosan in an aqueous solution of metal salts, organic or inorganic acid solution, complex with the corresponding compound can be easily prepared (Cairns, Miles, Morris, Ridout, Brownsey & Winter, 1992; Demarger-Andre & Domard, 1994; Kawadda, Abe, Yui, Okuyama & Ogawa, 1999; Ogawa & Inukai, 1987; Ogawa et al., 1993; Yamamoto, Kawada, Yui & Ogawa, 1997). The main chain conformations of these chitosan complexes are classified into two groups based on their X-ray diffraction patterns. One is the extended two-fold helical structure with about 10 Å axial repeat (Type I) and the other is the so-called "eight-fold" (8/3 or 8/5) helical structure with 40 Å axial repeat (Type II) which is a relaxed version of the two-fold helix. This classification is compatible with the two types proposed for chitosan depending on the single or double splitting of the C_1 carbon atom in their solid-state ^{13}C NMR spectra (Saito, Tabeta & Ogawa, 1987). Complexes with monocarboxylic acids, such as formic acid and acetic acid are formed in the solid state and they

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0144-8617/00/$ - see front matter
PII: S0144-8617(99)00142-3

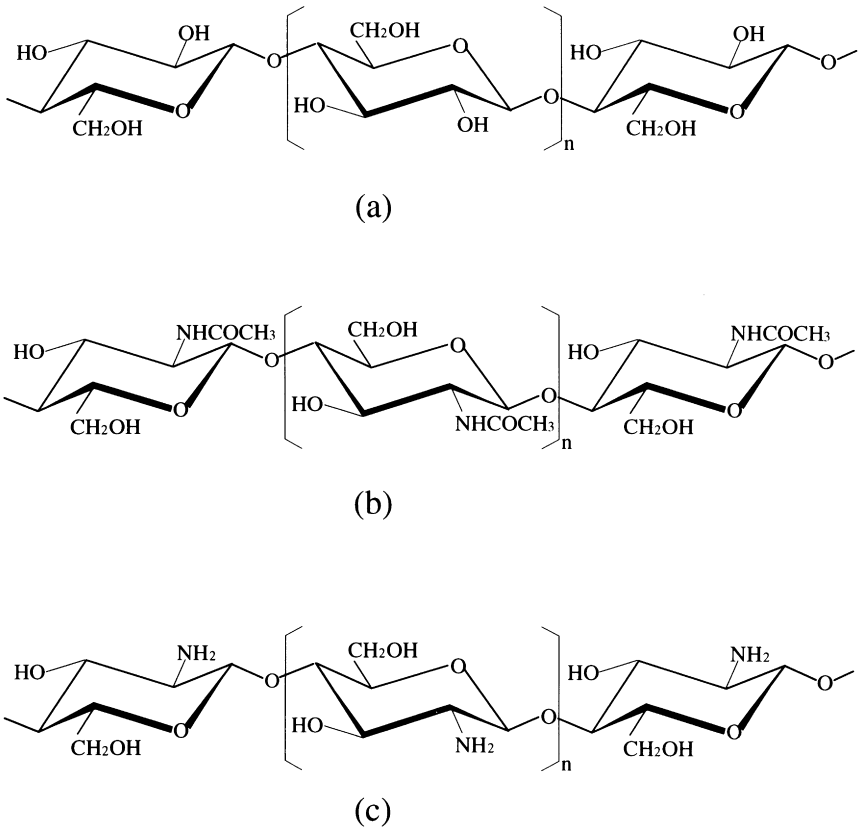

Fig. 1. Similarities of chemical structures of: (a) cellulose; (b) chitin; and (c) chitosan.

show interesting features. They change their molecular conformation from the extended two-fold helical structure with 10.34 Å axial repeat in the hydrated form to the eight-fold structure (Cairns et al., 1992; Ogawa & Inukai, 1987) with 40 Å axial repeat. Subsequently, the eight-fold structure changes to the two-fold structure of the anhydrous form with the passage of time. The time needed for this transformation depends on the monocarboxylic acid used for the complex formation and relative humidity of the specimen (Kawadda et al., 1999). This anhydrous form has definitely better orientation and crystallinity than that obtained by the annealing method. These features could be improved even more by exposing the specimen to the acid vapor instead of immersing the specimen in solution (Fig. 2). The resulting anhydrous form gave more reliable structural details (Okuyama et al., 1999b) than that obtained by the annealing method (Yui et al., 1994).

In this paper, molecular and crystal structures of the hydrated and anhydrous form of chitosan and those of Type I and II chitosan complexes will be discussed. Especially, the difference and transformation between the two-fold helix and the eight-fold helices will be focussed.

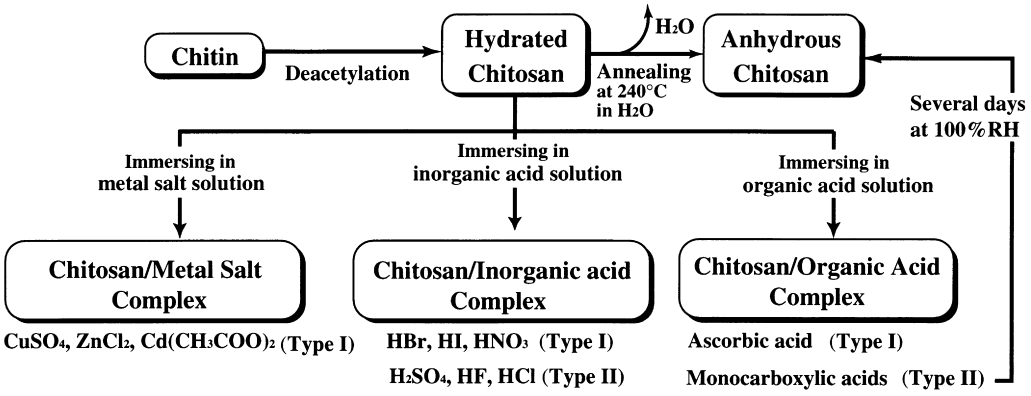

Fig. 2. Structural diversity of chitosan and its complex.

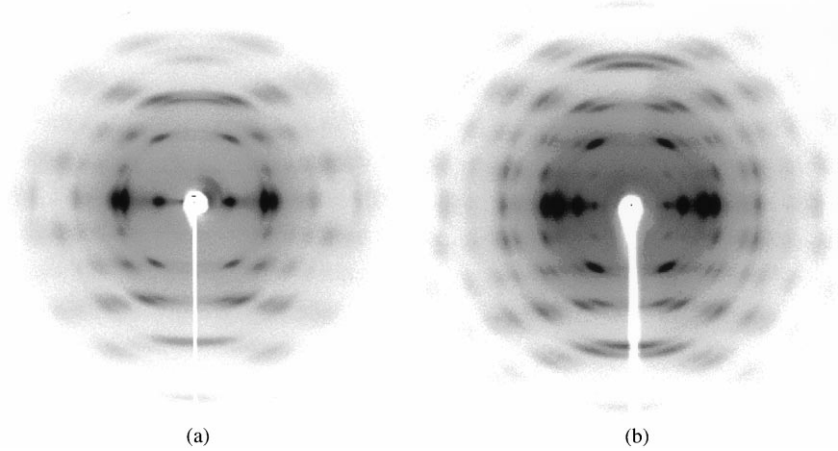

Fig. 3. X-ray fiber diffraction patterns of: (a) hydrated form of tendon chitosan; and (b) anhydrous form of tendon chitosan prepared via chitosan/acetic acid complex.

2. Materials and methods

2.1. Sample preparation

A tendon chitosan was prepared from the chitin of a crab tendon, *Chionoecetes opilio* O. Fabricius, by *N*-deacetylation with 50% sodium hydroxide solution at 110°C for 2 h under nitrogen atmosphere, after overnight removal of inorganic compounds with dilute HCl solution. The deacetylation procedure was repeated twice to achieve a complete conversion. The degree of *N*-acetylation by the colloidal titration and the viscosity average degree of polymerization of the tendon chitosan were found to be 0% and 10,800, respectively (Ogawa, 1991). This was used as the hydrated chitosan specimen (Fig. 2).

A part of the above specimen was immersed in a mixture of 4 M acetic acid and isopropanol (1/3 by v/v) for 3 h at room temperature or exposed to acetic acid vapor for several days at room temperature to prepare the chitosan/acetic acid complex. The formation of the complex was examined by X-ray diffraction pattern. Then the complex was kept in 100% RH for several days to remove the acetic acid and water in the crystal, so that the specimen changed to the anhydrous form. This specimen was used as the anhydrous chitosan (Fig. 2). In order to get the Type II complex, formic acid was used instead of acetic acid, since the former complex is stable compared with the latter.

In order to get chitosan/transition metal complexes, the hydrated tendon was pretreated by immersing in methyl alcohol and water to improve its accessibility to a metal solution (Ogawa et al., 1993). Approximately 4 mg of the tendon chitosan was immersed in an aqueous solution (30 ml) of metal salt, such as $CdSO_4$, $CdCl_2$, $Cd(NO_3)_2$ and $ZnCl_2$, at room temperature for about 3–12 h, followed by washing with water and drying in air. The optimum concentration of the metal salt and pH, and immersion period of the tendon chitosan in solution were surveyed for each metal salt by taking fiber diffraction patterns of specimens prepared under systematically varied conditions.

To get chitosan/inorganic acid complexes, the hydrated tendon chitosan was immersed in 7 M HNO_3 aqueous solution for 10–20 min with stirring at room temperature, which was then washed with water and dried in air (Ogawa & Inukai, 1987). The specimen with higher orientation and crystallinity was obtained by exposing the specimen to HNO_3 vapor, while applying tension by weight for 3 days at room temperature.

2.2. X-ray diffraction

The X-ray diffraction patterns were recorded by a camera system equipped with an imaging plate (DIP-100S, MAC Science Co. Ltd) by using the graphite-monochromatized CuKα radiation (1.5418 Å) or MoKα radiation (0.7107 Å) from an X-ray generator (ultraX18, Rigaku Co. Ltd) operated in normal focus mode with 50 kV and 250 mA. The in-house data processing software (Obata & Okuyama, 1992; Obata & Okuyama, 1994) was used to obtain the X-ray intensity for each diffraction spot and to determine the unit cell parameters.

2.3. Thermal analysis

To remove water trapped in the microvoid, the specimen was first immersed in acetone to exchange water by acetone. To remove acetone in the microvoid completely, the specimen was kept in a vacuum desiccator at 50°C for 30 min. The specimen was then examined by thermogravimetry as well as differential thermal calorimetry (THERMO PLUS TG 8110, Rigaku Co.) at a scanning rate of 10°C min^{-1} from room temperature to 300°C under nitrogen atmosphere. The specimen, just before thermogravimetry, was X-rayed to confirm its crystalline form.

Table 1
Crystal data of chitosan and its complexes

	Anhydrous chitosan	Hydrated chitosan	Chitosan/HNO_3	Chitosan/$ZnCl_2$	Chitosan/$CdCl_2$	Chitosan/$CdSO_4$	Chitosan/$Cd(NO_3)_2$	Chitosan/HCOOH
a (Å)	8.26(2)	8.95(4)	9.57(4)	10.31(2)	9.88(4)	10.61(3)	9.56(4)	10.58
b (Å)	8.50(1)	16.97(6)	18.64(6)	11.70(4)	11.97(4)	12.00(5)	12.8(1)	10.85
c (fiber axis) (Å)	10.43(2)	10.34(4)	10.40(3)	10.35(1)	10.36(2)	10.35(2)	10.33(2)	40.8
β (°)	90.0	90.0	90.0	122.3(1)	115.2(2)	114.0(2)	115.4(3)	90.0
Volume $\times 10^{-2}$ ($Å^3$)	7.32	15.70	17.46	10.54	11.08	12.03	11.46	46.84
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_1$	–	–	–	*P1*
Number of spots	41	24	39	41	37	29	11	53
D_{obs} (g cm^{-3})	1.42	1.45	1.58	1.66	1.80	1.79	1.79	1.44
D_{cal} (g cm^{-3})	1.52	1.52	1.70	1.66	1.83	1.88	1.64	–
Number of chains passing through the cell	2	4	4	2	2	2	2	2
Cross-section of chain ($Å^2$)	35.1	37.9	42.2	50.9	53.5	58.1	55.2	57.3

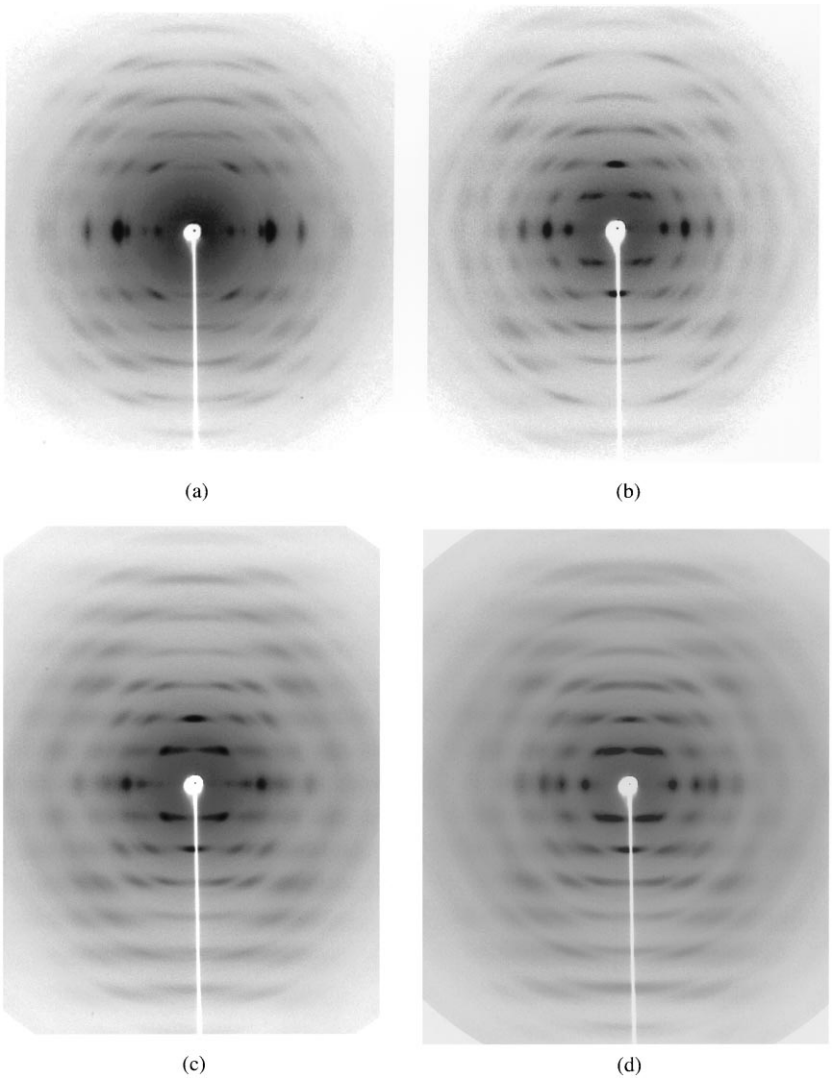

Fig. 4. X-ray diffraction patterns of Type I form: (a) HNO_3 salt; (b) $ZnCl_2$ salt; (c) $CdCl_2$ salt; and (d) $CdSO_4$ salt.

2.4. Density measurement

In order to remove air trapped in the microvoid of the specimen, the specimen was immersed in tetrachloromethane and degassed by using an aspirator. Density was measured by the flotation method by adding a solvent, such as toluene, dibromoethane and dichloroethane, to tetrachloromethane.

3. Results and discussion

3.1. X-ray diffraction and lattice parameters

Although the details of the X-ray diffraction patterns (Fig. 3) of the hydrated (Okuyama et al., 1997) and anhydrous (Okuyama et al., 1999a) tendon chitosan are different, their fiber repeats are essentially the same ($c = 10.3$ Å), indicating that the chains have a fully-extended two-fold helical conformation in both cases. Lattice dimensions of these two forms are shown in Table 1. So far, several lattice parameters were proposed for various chitosan specimens (Clark & Smith, 1937; Ogawa et al., 1984; Sakurai, Takagi & Takahashi, 1984; Yui et al., 1994) including single crystals of chitosan oligomer (Cartier, Mazeau, Domard & Chanzy, 1992). These were classified into two, hydrated and anhydrous forms. In the former case, all the lattice parameters of chitosan ($a = 8.9$; b (fiber axis) $= 10.25$ and $c = 17.0$ Å) from lobster tendon (Clark & Smith, 1937) and those (Table 1) from crab tendon (Okuyama et al., 1997) are essentially the same, but the b-dimension in column 3 of Table 1 is twice that of c ($a = 8.67$; b (fiber axis) $= 10.24$; $c = 8.92$ Å, $\beta = 92.6°$) proposed by Sakurai et al. (1984). In their study, regenerated films from chitosan powder were used as specimens which had poor orientation and poor crystallinity compared with tendon chitosan specimens. Since the intensity distribution in its fiber diffraction pattern was quite similar to that of

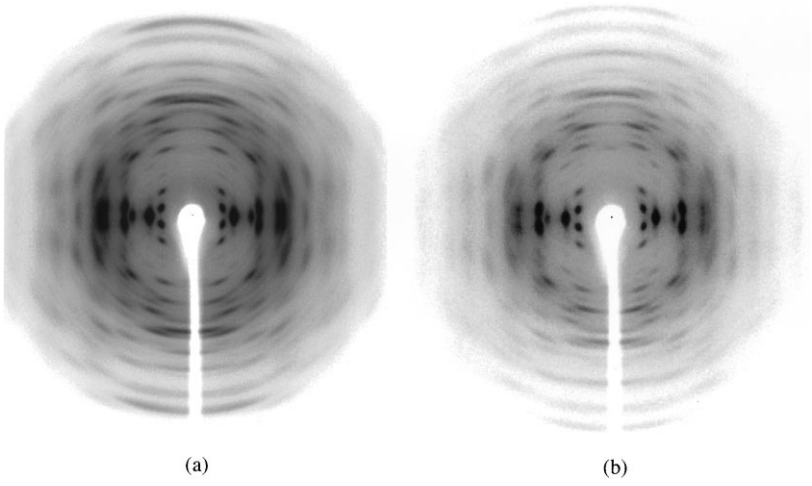

Fig. 5. X-ray diffraction patterns of: (a) chitosan/HCOOH; and (b) chitosan/acetic acid.

tendon chitosan, and also every diffraction spot could be explained by the one obtained from crab tendon, the regenerated film seems to be essentially isomorphous as that of tendon chitosan. In the case of anhydrous chitosan, the lattice parameters ($a = 8.28$; $b = 8.62$; and $c = 10.43$ Å) of the specimen produced by the annealing method from hydrated tendon chitosan (Yui et al., 1994) are similar to those obtained via chitosan/acetic acid complex (Table 1), and those from the single crystal of chitosan oligomer ($a = 8.07$; $b = 8.44$; and $c = 10.34$ Å) in an electron diffraction study (Cartier et al., 1992; Mazeau, Winter & Chanzy, 1994).

Fiber diffraction patterns of chitosan/metal salts complexes (Fig. 4b–d) also shows similar features. That is, in addition to the overall intensity distribution, they have a similar axial repeat of 10.3 Å and a fairly strong 002 reflection. This group of complexes has similar lattice parameters (Table 1) and hence essentially the same packing arrangement having a fully extended two-fold helical structure with about 10.3 Å axial repeat. Besides those listed in Table 1, several other unit cells could explain the observed X-ray data: for example, the larger unit cell once proposed for a series of chitosan/metal complex (Ogawa et al., 1993). Recently, we have obtained smaller unit cells for every chitosan/metal complex, in which each is slightly different from others due to the metal salt (Table 1). As shown later, one of them, chitosan/$ZnCl_2$ complex, has been analyzed intensively, and a reasonable crystal structure obtained. All the crystal structures of metal complexes studied so far belong to Type I. On the other hand, chitosan/inorganic or chitosan/organic acid complex are of both Types I and II depending on the kind of acid.

Fiber diffraction patterns of chitosan/HNO_3 complex shown in Fig. 4 are very similar to that of the hydrated form (Fig. 3a). Ogawa and Inukai (1987) found that the unit cell parameters of the chitosan complexes with various inorganic acid are similar to those of hydrated chitosan when they adopted Type I. The chitosan/HNO_3 complex has the best orientation and crystallinity. Although alternate unit cells are plausible, the one listed in Table 1 has led us to a reasonable crystal structure.

Contrary to the Type I complexes, the diffraction patterns of Type II complexes (Fig. 5) are very similar, which show that they are independent of the kind of acid. They all have a long axial repeat of 40 Å. For each, two or more unit cells are possible. One of them is that proposed for chitosan/HCl complex (Ogawa & Inukai, 1987), and it has $a = 13.81$; $b = 16.33$; c (fiber axis) $= 40.73$ Å and $\gamma = 96.46°$. This cell resembles those for the other complexes with inorganic acids, such as HF and H_2SO_4 (Ogawa & Inukai, 1987), and with acetic acid (Cairns et al., 1992). However, according to our recent study of the chitosan/HCOOH complex, 53 observed spots could be explained by an alternate unit cell ($a = 10.58$; $b = 10.85$; c (fiber axis) $= 40.8$ Å) (Table 1).

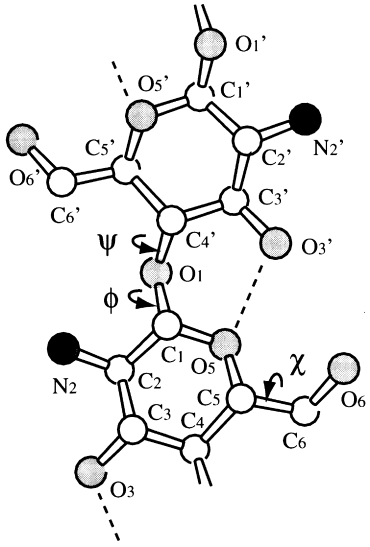

Fig. 6. Chemical structure of a disaccharide segment of chitosan together with atomic numbering. The conformational angles, ϕ and ψ, which define the molecular conformation, are also shown.

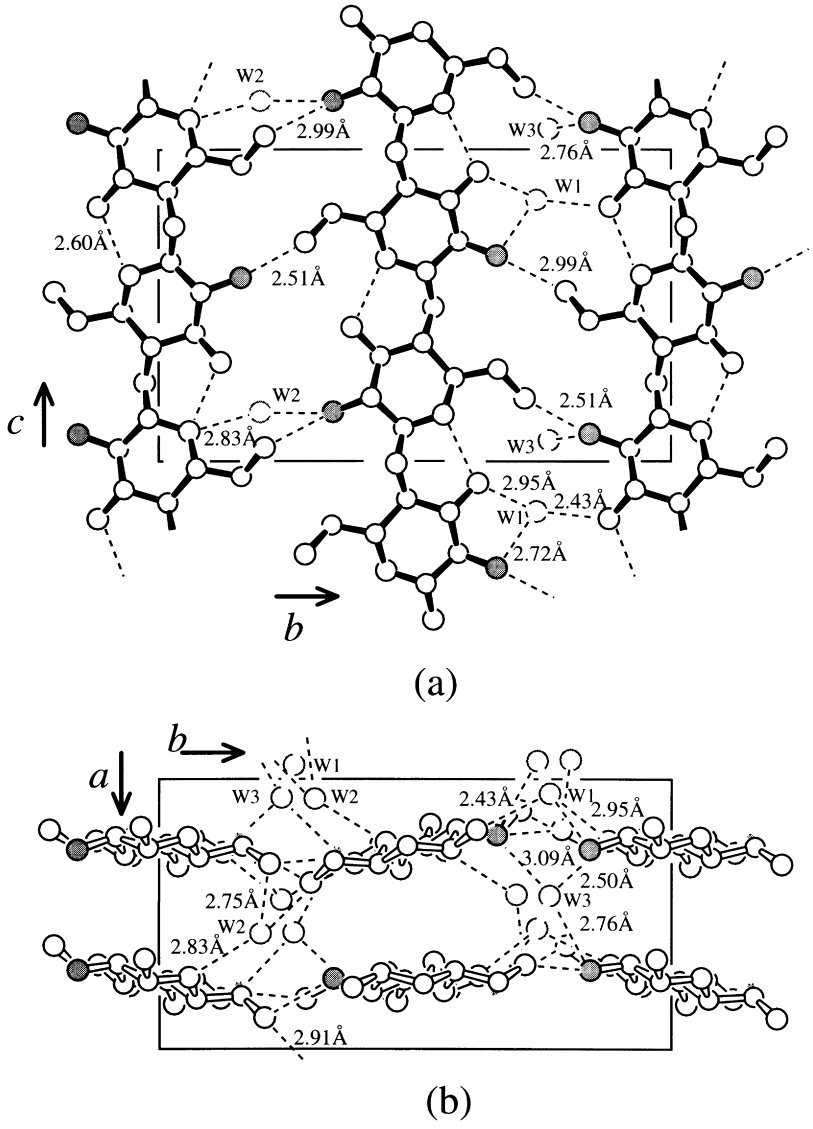

Fig. 7. Packing arrangement of hydrated chitosan in the unit cell projected (a) along the *a*-axis and (b) along the *c*-axis. Gray circles denote nitrogen atoms. Only one of the two sheets parallel to the *bc*-plane is shown in (a) for the sake of clarity. The chains on the *a*-axis in (b) are up-pointing while the others are down-pointing.

Since the fiber diffraction patterns of formic acid salt and acetic acid salt are similar (Fig. 5), this unit cell must also be valid for the acetic acid salt. On this basis, a reasonable crystal structure is now available (Okuyama et al., 1999b).

3.2. Crystal structures of hydrated and anhydrous chitosan

The polymer chains in both hydrated and anhydrous forms have two-fold helical symmetry with extended fiber repeat of about 10.3 Å and are reinforced by the $O_3\cdots O_5$ hydrogen bonds (atomic numberings are shown in Fig. 6). This is the typical structure of the β(1 → 4) linked polysaccharides such as cellulose, mannan and chitin. In the chitosan structures reported (Okuyama et al., 1997; 1999b), dihedral angles ϕ (C_2–C_1–O_1–C_4') and ψ (C_1–O_1–C_4'–C_3') are 145.9 and 94.1° for the hydrated form, and 146.0 and 93.8° for the anhydrous form, respectively. Atom O_6 has a *gt* conformation ($\chi(O_5$–C_5–C_6–$O_6) = 68.6°$ for the hydrated and 48.8° for the anhydrous forms). Therefore, the molecular conformations of hydrated and anhydrous chitosan are conserved.

In hydrated chitosan, there are four polymer chains passing through the unit cell. The two adjacent chains along the *b*-axis are crystallographically independent (Fig. 7), arranged in an antiparallel fashion and linked by two sets of $N_2\cdots O_6$ hydrogen bonds (2.51 and 2.99 Å), so as to form a sheet structure parallel to the *bc*-plane (Fig. 7a). These sheets are piled up along the *a*-direction (Fig. 7b). As a result, two independent polymer chains running in the same direction from the asymmetric unit along the *a*-axis. Although there is room for three water molecules, X-ray agreement, observed density and thermogravimetric

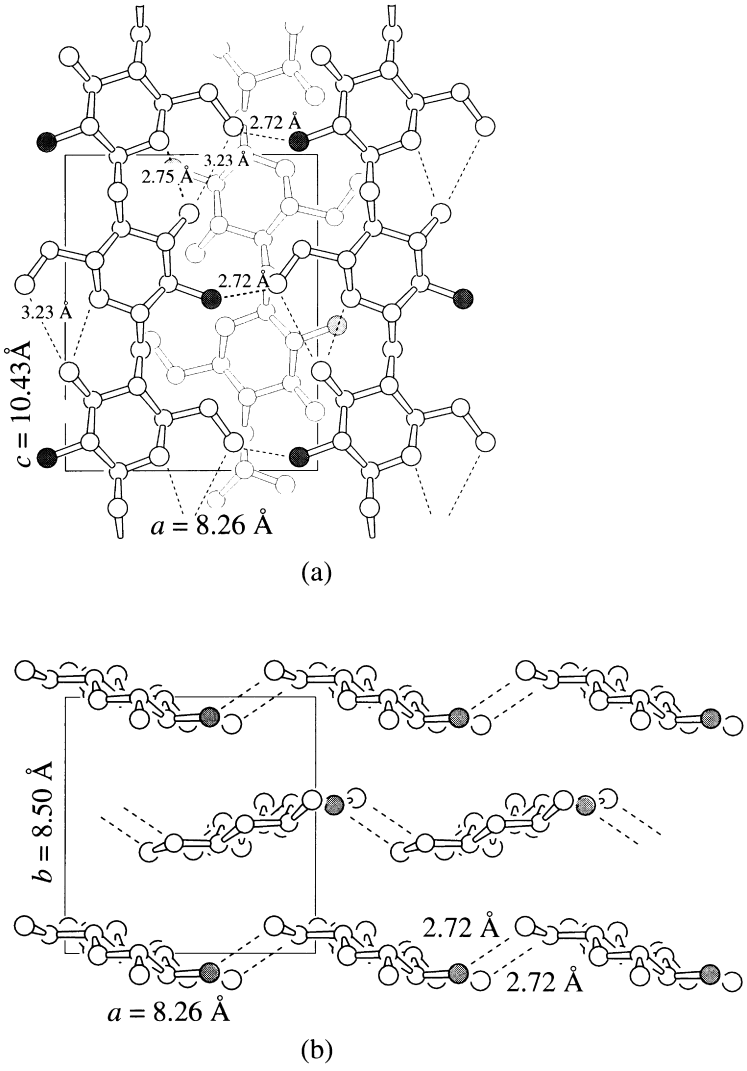

Fig. 8. Packing arrangement of anhydrous chitosan projected (a) along the *b*-axis and (b) along the *c*-axis. Gray circles denote nitrogen atoms. The chains in the upper and lower sheets in (b) are down-pointing while those in the middle sheet are up-pointing.

analysis identify only two water molecules in an asymmetric unit. This rather loose packing arrangement may facilitate complex formation with various acids and transition metals in the solid phase.

In anhydrous chitosan, two adjacent polymer chains along the *a*-axis are linked by a series of $N_2\cdots O_6$ hydrogen bonds (2.72 Å), which make the sheet structure parallel to the *ac*-plane (Fig. 8). Neighboring sheets are related by crystallographic 2_1-symmetry along the *a*-direction, which result in an antiparallel stacking of the sheets along the *b*-direction (Fig. 8b). Within the sheet, the short contacts (less than 4 Å) between adjacent chains are $C_6\cdots N_2 = 3.56$ and $C_2\cdots O_6 = 3.92$ Å. There is no hydrogen bond between adjacent sheets, and the short contacts are $C_1\cdots O_6 = 3.44$, $N_2\cdots O_1 = 3.51$, $C_4\cdots O_6 = 3.60$, $C_3\cdots O_3 = 3.66$, $N_2\cdots O_5 = 3.67$ and $O_3\cdots O_6 = 3.77$ Å. Similar features with only van der Waals contacts between adjacent sheets have been found in plate-like polymers such as poly(*p*-benzamide) (Okuyama et al., 1989) and cellulose I (Gardner & Blackwell, 1974).

3.3. Plausible crystal structure of Type I chitosan complex

From a 10.3 Å axial repeat observed in the fiber diffraction patterns of chitosan/HNO_3 and chitosan/metal salts, we can assume that the polymer conformation in Type I is a fully extended two-fold helical structure as in the hydrated and anhydrous forms of chitosan. Although complete structure analyses of these complexes are not finished yet, a plausible model for chitosan/HNO_3 complex is shown in Fig. 9. The final result of this analysis will be published later elsewhere. Here, we shall look at the essence of the packing arrangement.

Polymer chains in the chitosan/HNO_3 crystal are located at the special position ($u = 0.25$, $v = 0.0$) and ($u = 0.75$, $v = 0.0$), in space group $P2_12_12_1$, the molecular 2/1-helical axis

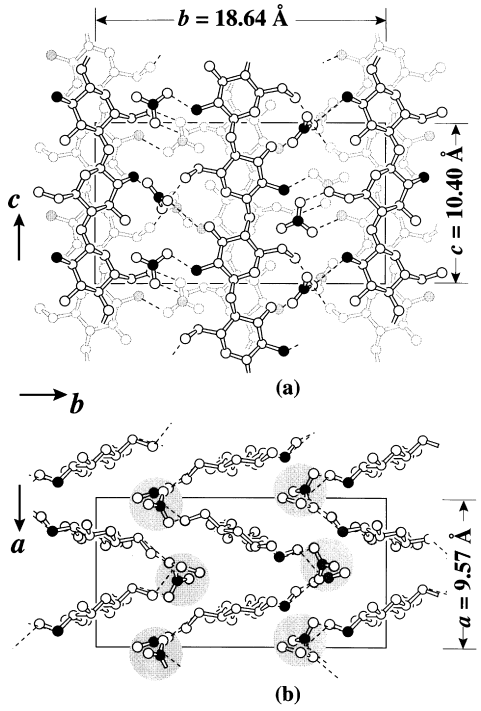

Fig. 9. Plausible packing structure of chitosan/HNO_3 complex projected (a) along the *a*-axis and (b) along the *c*-axis.

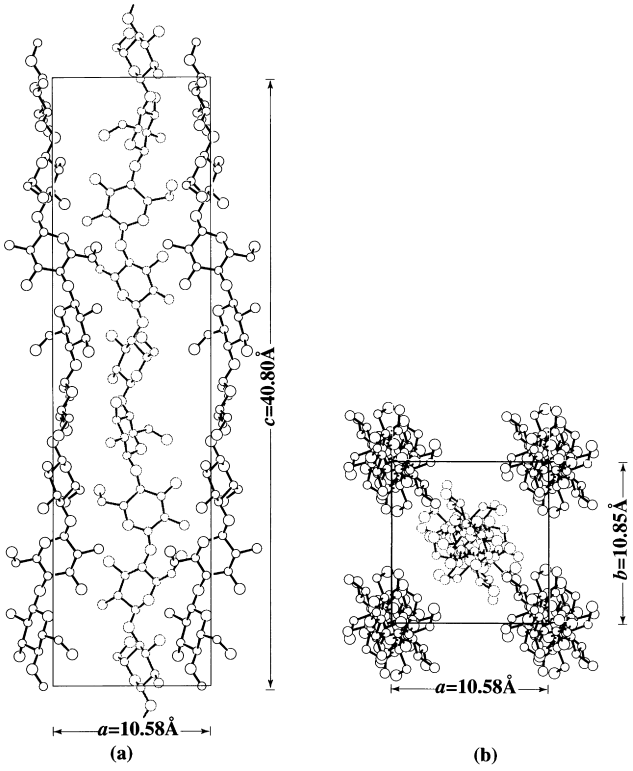

Fig. 10. Plausible parallel packing structure of chitosan molecules in the Type II form projected (a) along the *b*-axis and (b) along the *c*-axis. Chains at ($u = 1/4$ and 3/4, $v = 0$) have the same polarity but opposite to that of the other two chains.

coinciding with the crystallographic 2_1 axis along the *c*-axis. This setup makes these two polymer chains independent and leads to two possibilities. In the first, the two chains point the same direction (parallel model), while in the second, two chains point opposite directions (antiparallel model). In both cases, acid molecules can be located on both sides of the polymer chain and they connect adjacent polymer chains along the *a*-axis by hydrogen bonds to form a zigzag arrangement of chains in this direction (Fig. 9b). Since the up-pointing polymer chains are aligned along the *a*-axis in the hydrated form (Fig. 7b), the parallel model will be easily accessible to HNO_3 as shown in Fig. 9b. On the other hand, to generate an antiparallel model, alternate polymer chain along the *a*-axis should reverse its polarity. It is unthinkable that such a change would be easy as the hydrated form transforms to the HNO_3 complex. However, such a model is under investigation.

In the chitosan/metal salt complexes, the unit cell contains four glucosamine residues. Water contents of chitosan/$ZnCl_2$, chitosan/$CdCl_2$, chitosan/$CdSO_4$ and chitosan/$Cd(NO_3)_2$ are found to be 13, 17, 22 and 12 wt%, respectively, from thermogravimetric measurements. Using this information, and for 1:1 molar ratio of glucosamine residue to metal salt, the calculated densities in the four cases are 2.15 (1.87), 2.49 (2.06), 2.62 (2.04) and 2.21 (1.95), respectively. Note that the values in parentheses correspond to the calculated density without water molecules. The calculated densities (D_{cal}) for the molar ratio 2:1 are listed in columns 5–8 in Table 1. Comparison between calculated and observed densities suggest that the 2:1 molar ratio is appropriate, while 1:1 is not. This result is consistent with that reported from atomic absorption spectrophotometry (Ogawa et al., 1993).

3.4. Packing arrangements in Type II chitosan complex

Although an 8/5-helical model with one glucosamine as a helical asymmetric unit has been proposed as a favorable molecular conformation for Type II chitosan/monocarboxylic acid complex (Cairns et al., 1992), we found recently that this model does not agree with the X-ray diffraction data at all. Instead, a relaxed two-fold helix with a tetrasaccharide repeat ($c = 40.8$ Å) in a helical asymmetric unit, is satisfactory (Okuyama et al., 1999b). The results show that there are two antiparallel polymer chains, one at the corner and the other at the center of the unit cell projected along the *c*-axis. The shape of the molecule is like a cylinder when projected along the *c*-axis (Fig. 10b). The cross-section of the chain is about 57.3 $Å^2$, which is quite large compared with other chitosan complexes and chitosan itself (Table 1). The space between chitosan chains is filled with water and carboxylic acid, which account for more than 33% by weight according to thermo-gravimetric measurement and the observed density. There should be some reason why a less-extended helix with tetrasaccharide repeat in the asymmetric unit is preferred to the fully extended two-fold

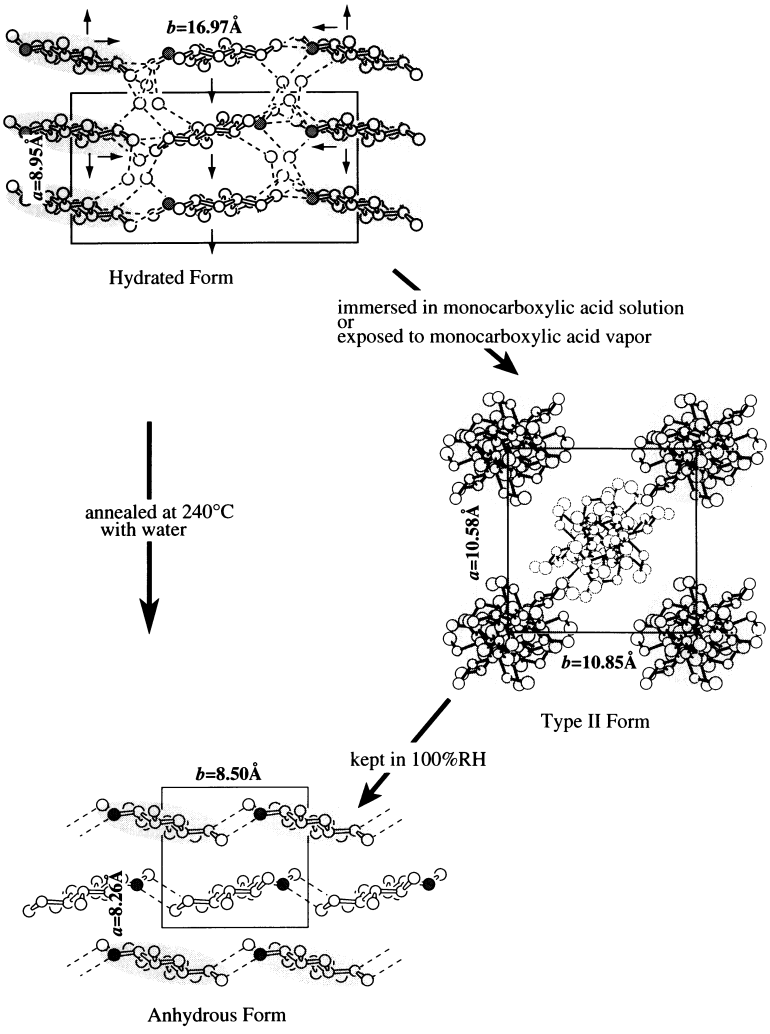

Fig. 11. Speculation of crystal–crystal transformation. Molecules with gray color are up-pointing, while the others are down-pointing.

helix with a monosaccharide repeat observed in all other cases. The details of interaction between polymer chain and monocarboxylic acid seem to be the answer at this stage.

3.5. Speculation of crystal–crystal transformation

Since the transformation from the hydrated form to the anhydrous form takes place in the solid state via the chitosan/monocarboxylic acid complex, it is important to understand the pathway. Especially, the polymer chain conformation in Type II may have some role, as an intermediate structure. This would be the case even in the annealing method where the elevated temperature and pressure might cause similar conformational changes in the solid state.

Following is a speculation of the steps involved in the transitions. When hydrated chitosan specimens are immersed in a mixture of monocarboxylic acid and isopropanol, the acid permeates into the specimen through water columns along the *c*-axis in the hydrated chitosan unit cell (Fig. 11). Then, the interactions between the polymer chain and the acid force the hydrogen bond network among polymer chains and water molecules to break, thereby facilitating the conformational change from the extended two-fold helix to the less-extended relaxed two-fold helix of the complex. During the transformation to Type II, all the down-pointing chains (unshaded) in the hydrated form must shift 0.25 along the *a*-axis by breaking the hydrogen bonds between neighboring polymer chains. At the same time, the shrinkage of the lattice along the *b*-direction and the enlargement along the *a*-direction take place to form the alternate repetition of up-pointing and down-pointing chains in the complex structure.

The cross-section of a polymer chain in the complex structure is about 60% larger than that of the anhydrous form. This space is filled with formic acid and water molecules. Chitosan chain seems to be isolated in this space, which may cause the pseudo-tetragonal packing of the chitosan/monocarboxylic acid complex. Although the

complex structure is stable in the acid rich surroundings, the chitosan conformation changes to the original extended two-fold helical structure by the spontaneous removal of acid and water in the acid free surroundings. The reason for this spontaneous removal is not clear but may be attributed to the higher stability of the extended two-fold helix over the less-extended, relaxed two-fold helix of the chitosan chain. That is, the extended two-fold helical conformation, reinforced by the hydrogen bond between O_3 and O_5, is very stable and is observed in most of the $\beta(1 \rightarrow 4)$ linked polysaccharide structures under conditions with or without water. On the other hand, in the chitosan/HCOOH complex, not all the intrachain $O_3 \cdots O_5$ hydrogen bonds would be equally strong because of the conformational variability in the tetrasaccharide motif, but the overall structure could be stabilized only by favorable interactions with the acid. Therefore, if there is not enough acid molecules in the surroundings, the relaxed conformation cannot be sustained and it will revert to the stable extended structure. At present, the structural details of chitosan/HCOOH are tentative, and hence the preferred interactions between chitosan and formic acid molecules are not known. We also do not know why the removal of HCOOH is accompanied by dehydration and how this is facilitated by 100% relative humidity only.

4. Conclusion

Chitosan and its complexes prepared from crab tendon chitin in the solid state were studied by X-ray diffraction method. Since crab tendon chitin is a highly oriented and crystalline material, the derived specimens are also highly oriented. Relevant lattice parameters for different chitosan specimens reported so far could be classified into two groups, as the hydrated and anhydrous forms. Their structures have been determined by using the above specimens. In these structures, the molecular conformations are essentially the same, while the packing arrangements and water contents are quite different from each other.

Chitosan complexes with acid or metal salts can be classified into two groups depending on the axial repeat along the fiber axis. Type I has a 10.3 Å axial repeat similar to the hydrated and anhydrous chitosan, while Type II has a 40 Å axial repeat. In all complexes studied, new cell parameters have been proposed by using the above specimens and an imaging plate. These cell parameters seem to be reasonable since plausible crystal structures have been obtained in each case. The Type II conformation is found to be a less-extended two-fold helix with a tetrasaccharide in the asymmetric unit rather than the prevailing 8/5-helical model.

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