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Conformational Diversity of Chitosan

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Abstract

Three crystalline polymorphs of chitosan have been analyzed by x-ray diffraction method. First, the most abundant, is called 'tendon chitosan' polymorph, which is a hydrated form and has been prepared from of a crab tendon chitin by a solid-state N-deacetylation. Second, another hydrate crystal called 'L-2' was obtained with a chitosan film prepared from the liquid crystal solution. Third, 'annealed chitosan', obtained by heating the 'tendon chitosan' in the presence of water at a high temperature around 200°C, is anhydrous. In these three polymorphs chitosan molecules take up similar conformation, an extended two-fold helix (Type I form) which is stabilized by intramolecular O(3)---O(5) hydrogen bond and is similar to the conformations of chitin and cellulose.

In addition to Type I form other three chitosan conformations have been found in the crystals of chitosan-acid salts. In the salts with acetic acid and other acids, so called Type II salt, chitosan molecule takes up a relaxed two-fold helix composed of an asymmetric unit of tetrasaccharide (Type II from). Chitosan molecule in its HI salt, which is prepared at low temperature, takes a 4/1 helix with asymmetric unit of disaccharide. The fourth chitosan conformation was most recently found to be a 5/3 helix that was observed on fiber diagrams of chitosan salt with medical organic acids having phenyl group such as salicylic or gentisic acids. A solid-state ^{13}C NMR study suggested that chitosan in the aspirin (acetylsalicylic acid) salt also takes the 5/3 helix.

Type II form seems to be unstable because no strong intramolecular hydrogen bond like that in the extended two-fold helix is present, and it is considered to be stabilized by acid ion of the salt. All of Type II salts change to the 'annealed chitosan' polymorph (Type I form) by spontaneous removal of the acid accompanied by water molecules when the salts were allowed to stand or immersed in an isopropanol-water mixture.

The polysaccharides consisting of β-(1→ 4) linked glucopyranose residues are known to be cellulose, chitin, and chitosan. The former two polymers normally exhibit an extended two-fold (zigzag) helix similar to that of Type I form of chitosan. Na cellulose, which is prepared by immersing cellulose in NaOH solution of a high concentration (18% or more), represents a 3/2 helix. In contrast, without such a strong atmosphere, chitosan can easily change its conformation from a zigzag structure (2/1 helix) to a relaxed two-fold, 4/1, or 5/3 helical conformations by normal neutralizations (salt formations). This conformational flexibility is advantage of chitosan to its advanced usage.

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Introduction

Molecular conformations of chitin and chitosan in solid state have been analyzed mostly by x-ray diffraction analyses on their crystals. Numerous crystallographic studies on chitin had been performed, and the molecular conformations recognized today are those analyzed by Blackwell and his coworkers published around 1970s. In contrast, studies for chitosan have been much less although the first x-ray fiber pattern was published in 1936 by Clark and Smith. The complete analyses for chitosan fiber patterns were done in the1990s; almost 60 years later! This time difference of analysis between chitin and chitosan is owing to their natural abundance: the former is the second most abundant polysaccharide in the world next to cellulose, whereas chitosan is much less.

Studies on crystalline conformations of chitosan have rapidly been progressing depending on the requirement from the tremendous applications of chitosan in various purposes. In this manuscript, recent developments in the study on crystalline conformations of chitosan are introduced. And, a strange behavior of a chitosan acid salt called 'Spontaneous water removing action by acid' is described (Ogawa *et al.*, 2004).

Chitosan crystals

Three crystalline polymorphs of chitosan have been analyzed, so far. One is called 'tendon chitosan' prepared from crab tendon chitin by a solid state *N*-deacetylation, which has originally been obtained by Clark and Smith (1936).

In this crystal, fiber axis length was 1.04 nm, and chitosan chain takes an extended two-fold helix, in other word, a zigzag structure, which is stabilized by intramolecular O(3)---O(5) hydrogen bond. This conformation is called Type I form (Figure 1). Neighboring chitosan chains are packed in an antiparallel fashion in the crystal, and chitosan chains showing opposite direction are bonded by hydrogen bonds to make a sheet structure. This is a hydrated crystal where water molecules are present between these sheets and stabilize this crystal structure. This polymorph is the most abundant, i.e., commercially available chitosan samples have this crystal although their crystallinity is different. Another hydrated crystal called 'L-2' was found by Sakurai *et al.* (1985) with a chitosan film prepared from the liquid crystal solution. In this polymorph each chitosan chain also takes an extended two-fold helix similar to that in 'tendon chitosan'.

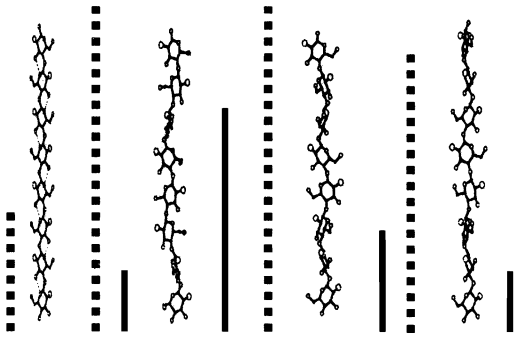
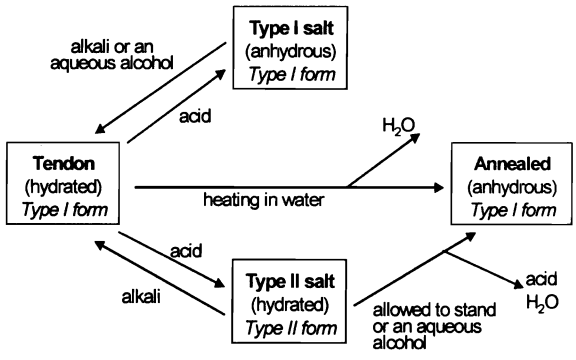


Figure 1. Conformational diversity of chitosan molecule. Large circle are nitrogen atoms, and small, oxygen atoms. Dotted lines are repeating units, and straight lines, asymmetric units. Small dotted lines in Type I formare O(3)---O(5) intramolecular hydrogen bonds.

When tendon chitosan was heated in the presence of water at around 200°C, the resultant chitosan specimen gave another x-ray fiber pattern called 'annealed chitosan' polymorph. This change in chitosan crystal is an irreversible process (Scheme 1). In this crystal, each chitosan chain takes an extended two-fold helix, zigzag structure, similar to that in tendon polymorph, and the chains are also packed in an antiparallel fashion. However, there is no water molecule in this crystal indicating that this is an anhydrous crystal. Different from previous tendon polymorph, neighbor chains having parallel direction are bonded by direct hydrogen bonds to make a sheet, and neighbor sheets having antiparallel direction are stacked. Similar crystal structure of this anhydrous crystal was also found by electron diffraction analysis (Mazeau *et al.*, 1994). Thus, only the zigzag structure has been found in chitosan crystals so far. This structure is also similar with those of chitin and cellulose.



Scheme 1. Crystalline transformation of chitosan

Chitosan-acid salts

In addition to Type I form (the extended two-fold helix) other chitosan structures have been found in acid salts of chitosan. Crystal structures of several chitosan salts with inorganic and organic acids have been studied, and they have been found to be classified into four types, depending not only on kind of acid but also on acid concentration or temperature at salt preparation (Table 1). Type I salts are mostly anhydrous, and in these crystals the backbone chitosan chains retain the extended two-fold helix of the unreacted chitosan molecule (Type I form). Conformational change of chitosan molecule occurred by salt formations of types II & III.

Table 1. Classification of chitosan acid salts

Type I	*An extended 2-fold helix (anhydrous)*	HNO_3 (high concn.), HBr, HI (at high temp.), $HClO_4$*, L- or D-lactic acid (at high temp.), maleic acid, L- ascorbic acid, D-isoascorbic acid, Salicylic Acid (at low temp.)
Type II	*A relaxed 2-fold helix (hydrated)*	HNO_3 (low concn.),H_2SO_4, HCl, HF, HIO_4*, H_3PO_4*, L- or D-lactic acid (at low temp.), succinic acid, fumaric acid, L-tartaric acid, monocarboxylic acids (formic, acetic, propionic)
Type IIa	*A 4/1 helix (hydrated)*	HI (at low temp.)
Type III	*A 5/3 helix (anhydrous)*	Salicylic Acid (at high temp.), Gentisic Acid, Aspirin*

*By Solid-State ^{13}C NMR

When tendon chitosan was immersed in a mixture of aqueous acetic acid and isopropanol, resulting salt gave a fiber pattern showing longer fiber axis of 4.08 nm: almost 4 times longer than that of Type I form (1.04 nm). This suggests the occurrence of a conformational change in chitosan molecule by the salt formation. The salt was called Type II. Different to Type I salts, Type II salts are hydrated crystals. Interestingly, all other Type II salts showed similar fiber patterns to that of the chitosan acetic acid salt indicating that they have identical unit cell dimensions in spite of the difference of acid. These facts suggest that anions were not present regularly in the respective crystals of the Type II salts and consequently that only the backbone chitosan chains contributed to the fiber pattern. The molecular structure of chitosan in the crystal of Type II salts is shown in the Figure 1, and this chitosan conformation is called Type II form. In this crystal, chitosan molecules are arranged in an antiparallel fashion, as well. Different to chitosan chain in Type I form, this chitosan molecule has longer fiber repeat, and asymmetric unit consists of 4 glucosamine residues, in other words, tetrasaccharide. Since two tetrasaccharides make a fiber repeat, this is also a two-fold helix although the conformation is different to that of Type I form where asymmetric unit is a glucosamine residue. Type II form is called relaxed two-fold helix.

Another Type II salt called Type II a salt has been found with chitosan hydrogen iodide salt prepared at low temperature (Table 1). Crystalline unit cell of this salt is different to Type II salt but its fiber axis is similar; 4.054 nm. The molecular conformation is a 4/1 helix with asymmetric unit of glucosamine dimer residue, i.e. a right-handed helix consists of four asymmetric units (Figure 1).

Recently, a new chitosan conformation called Type III salt has been found in the chitosan salt with medical organic acids having phenyl group: salicylic and gentisic acids. Their fiber patterns have fiber repeat of 2.55 nm, indicating that chitosan chain in these salts takes a five-fold helix with asymmetric unit of glucosamine residue. Searching possible five-fold helix using a computer program PS79 led that only a 5/3 helix, a left-handed helix with five glucosamine residues in two turns, was plausible. Aspirin having similar chemical structure to those of salicylic and gentisic acids is one of the most popular medicines. But, its chitosan salt did not show any x-ray diffraction pattern indicating no crystal. Thus, a solid-state NMR spectrum of the aspirin salt was performed. According to Saito *et al.* (1987) the ^{13}C chemical shifts of the C-1 and C-4 carbon atoms of chitosan and its acid salts, which are next to the glycosidic linkage, vary appreciably (up to 8 ppm) with the two torsion angles at the glycosidic linkages, C-1-Ogly (Φ) and Ogly-C-4 (Ψ), respectively. The chemical shifts of both C-1 and C-4 carbon atoms of the chitosan aspirin salt were similar to those of the gentisic acid salt, and are different from those of tendon chitosan. These indicated that chitosan molecule in the aspirin salt had a similar conformation (5/3 helix) to that in the gentisic acid salt.

The polysaccharides having similar chemical structure (β-1,4-linked polysac-charide) to chitosan are cellulose and chitin. Chitin conformation found so far is only an extended two-fold helix similar to type. I form of chitosan. Cellulose takes normally similar two-fold conformation as well. A soda-cellulose takes 3/2 helix, and some cellulose derivatives also

take other conformations than this two-fold helix. However, these cellulose derivatives are prepared under severe conditions, such as a strong alkali or chemical syntheses. Whereas, chitosan can easily change the conformation from the extended two-fold to other structures by salt formation with acids (Figure 1). Certainly, this is due to the presence of free amino group on the monomer residue of chitosan. This conformational flexibility is advantage of chitosan to its advanced usage.

Spontaneous transformation of chitosan conformation from Type II to Type I

A crystalline transformation has been observed with Type II salts called 'A spontaneous water-removing action of acids' by Demarger-Andre and Domard (1994) followed by Yamamoto *et al.* (1997). They have reported that anhydrous (annealed) chitosan crystal can be obtained at room temperature from chitosan salts of monocarboxylic acids by spontaneous removal of the acids accompanied by dehydration. In the case of chitosan acetic acid salt, the salt freshly prepared gave a fiber pattern of Type II salt. But, when this salt specimen was stored at room atmosphere for a long period, such as one month, the resultant specimen showed a completely different fiber pattern which was similar to that of the anhydrous (annealed) polymorph of chitosan. This spontaneous change was also observed with density measurements and infrared spectra. These facts indicate that, during the storage of the salt, all the acetic acids of the salt were spontaneously removed from chitosan molecules accompanied by water molecules. This resulted in formation of the anhydrous crystal of chitosan.

It has been considered that the spontaneous water-removing action of acid from its chitosan salt occurs in a monocarboxylic acid salt only. However, when other specimens of Type II salts, such as hydrochloric and L-lactic (low temperature polymorph) acid salts, were immersed in 75% aqueous isopropanol, they gave x-ray patterns similar to the anhydrous polymorph of chitosan. These facts suggest that the water removing action of acid occurs in chitosan salt not only with monocarboxylic acids but also with all other Type II salts.

On the other hand, Type I salts, where each chitosan molecule takes the extended two-fold helix, are stable. When a Type I salt was immersed in 75% isopropanol aqueous solution for a given time, however, the resultant specimen gave the similar fiber pattern to that of the original tendon polymorph of chitosan, indicating that the Type I salt returned to the hydrated chitosan of starting material.

Behaviors of Type I and II salts observed in the present study are illustrated in Scheme 1. The transformation from a chitosan salt to a hydrated polymorph of chitosan, observed in Type I salt, is natural, that is, all the acids dissociated from the salt in the presence of water. In contrast, the behavior of Type II salt is strange. What is driving force for this strange transformation? The extended two-fold helix of chitosan is stabilized by O(3)----O(5) intramolecular hydrogen bonds, whereas no intrachain hydrogen bond is observed in the relaxed two-fold helix. These facts suggest that the latter conformation is less stable than the former and it may be stabilized by the presence of anions in the salt. However, during storage (for the monocarboxylic acid salts) or immersing in the aqueous isopropanol (for all Type II salts), a spontaneous dissociation of acid is promoted, and the chitosan molecule tends to go to the more stable two-fold conformation, the annealed polymorph, by removing water molecules present between the chitosan chains.

The anhydrous crystal of chitosan highly crystallized is insoluble with any aqueous acid solution. It does not a make complex with any transition metal ion, and it may not be biodegradable. That is, chitosan molecule seems to lose these important functions in the anhydrous crystal. However, from another point of view, i.e., 'Getting Out of Oil Policy', the anhydrous crystal may serve as an inert resin alternative to, say, polytetrafluoroethylene although much more studies on the crystal are required. The 'annealed chitosan' crystal had not been able to obtain without any thermal decomposition since the crystal had been prepared by a heat treatment at high temperature such as 240°C. The spontaneous water-removing action of monocarboxylic acid in the chitosan salt, however, gave a new procedure to obtain the crystal without any thermal decomposition since the action occurs at room temperature.

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