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Plausible molecular and crystal structures of chitosan/HI type II salt

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Abstract—Chitosan/HI type II salt prepared from crab tendon was investigated by X-ray fiber diffraction. Two polymer chains and 16 iodide ions (I^-) crystallized in a tetragonal unit cell with lattice parameters of $a = b = 10.68(3)$, c (fiber axis) $= 40.77(13)$ Å, and a space group $P4_1$. Chitosan forms a fourfold helix with a 40.77 Å fiber period having a disaccharide as the helical asymmetric unit. One of the O-3···O-5 intramolecular hydrogen bonds at the glycosidic linkage is weakened by interacting with iodide ions, which seems to cause the polymer to take the 4/1-helical symmetry rather than the extended 2/1-helix. The plausible orientations of two O-6 atoms in the helical asymmetric unit were found to be *gt* and *gg*. Two chains are running through at the corner and the center of the unit cell along the *c*-axis. They are linked by hydrogen bonds between N-21 and O-61 atoms. Two out of four independent iodide ions are packed between the corner chains while the other two are packed between the corner and center chains when viewing through the *ab*-plane. The crystal structure of the salt is stabilized by hydrogen bonds between these iodide ions and N-21, N-22, O-32, O-61, O-62 of the polymer chains.

Keywords: Chitosan type II salt; Chitosan structure; Chitosan iodide salt; Fourfold conformation; Fiber diffraction

1. Introduction

Chitosan, a linear polymer of β-(1 → 4) linked-2-amino-2-deoxy-D-glucose, is derived from chitin, which is widely distributed in nature as the main component in the exoskeleton of crustaceans. This polymer has been recognized as a versatile material for decades[1] since it possesses useful inherent properties, among them low toxicity, ease of biodegradation, biocompatibility, and the ability to form chelates.[2] Although these properties depend on both the chemical structure and molecular conformation of chitosan, a few studies on the molecular conformation of chitosan have been so far reported.

Crystallographic studies of chitosan were originated by Clark and Smith in 1936.[3] In their studies, chitosan specimens were obtained by the solid-state deacetylation of crab tendon chitin, termed 'tendon chitosan'. An orthorhombic unit cell with dimensions $a = 8.90$, $b = 17.0$, and c (fiber repeat) $= 10.25$ Å was reported. Similar unit cell dimensions, $a = 8.95$, $b = 16.97$, and c (fiber repeat) $= 10.34$ Å, were also obtained from a precise structural analysis of crab tendon chitosan.[4] Tendon chitosan is a hydrated crystalline form of chitosan that can be converted to an anhydrous crystalline form by annealing[5] or transformation via some chitosan complexes.[6,7] The comparison with those of tendon chitosan, similar values for the *a*- and *c*-axes and one half of the *b*-value were observed in the anhydrous chitosan ($a = 8.26$, $b = 8.50$, and c (fiber repeat) $=$ 10.43 Å).[8] In both crystalline forms chitosan takes an extended conformation with a twofold helical symmetry and a fiber repeat of about 10 Å like chitin.

It is noteworthy that among related β(1 → 4)-polysaccharides such as cellulose and chitin, only chitosan complexes show a wide variety of conformations, while most complexes of others conserved an extended 2/1-helix. This is probably because chitosan exclusively contains a primary amino group. A regular distribution

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of amino groups in the structure enables chitosan to form complexes with transition metals as well as salts with some acids. Chitosan complexes obtained so far can be classified into three types based on their fiber repeats. The complexes with a fiber repeat of about 10 Å are called type I and are observed in the complexes with metal compounds such as $CuSO_4$, $ZnCl_2$, and $Cd(CH_3CO_2)_2$,[9] with inorganic acids such as HNO_3,[9,10] HBr,[10] and HI,[10] and with organic acids such as L- and D-ascorbic acid.[11] Extended twofold helical conformation was preserved in this type. On the other hand, the complexes with a fiber repeat of about 40 Å are classified as type II and are observed in chitosan salts with monocarboxylic acids,[6,9] HCl,[10,12] HF,[10] and H_2SO_4.[10] Favorable conformations for this type are found to be at least two: a relaxed 2/1-helix[13] and a 4/1-helix.[12] Recently, type III having a fiber repeat about 25 Å was reported for chitosan salts with salicylic and gentisic acids.[14] Their conformation was reported as a 5/3-helix. Both type I and type II salts could be formed in some acidic solutions such as those of L- and D-lactic acids, depending on preparation temperature.[15]

The anhydrous crystalline form with high crystallinity is found to be insoluble in any aqueous acid solution, to form no complex with transition metal ions, and to be difficult for biodegradation.[16] This crystalline form is not suitable for applications in which chitosan serves as biodegradable material within a short term. However, it shows potential for serving as fairly inert material. Some type II complexes, such as those from chitosan/monocarboxylic acids and chitosan/HCl, transform to this crystalline form during storage or by removing water and acid by immersing in 2-propanol.[6,7] Knowledge on the crystal structure of type II salt is needed for understanding this behavior. However, no detailed structure of type II has been reported.

The chitosan/HI salt obtained so far has a fiber repeat of about 10 Å and is classified as type I.[10] Recently, we obtained a diffraction pattern with a fiber repeat about 40 Å from this kind of salt.[12] This indicated that the chitosan/HI salt is one in which both types could be observed. However, the key factor of it assuming a different conformation is still uncertain. The diffraction patterns obtained from type II salts reported so far are similar to each other. Therefore, it is likely that chitosan molecules take a similar main chain conformation in type II salt crystals. A detailed structure determination of the chitosan/HCOOH salt showed that chitosan adopts a relaxed twofold helical conformation having a tetrasaccharide as a helical asymmetric unit.[13] The same conformation is also found in the chitosan/HCl salt.[12] However, the diffraction pattern with a fiber repeat of about 40 Å obtained from chitosan/HI salt showed a different intensity distribution from those of the relaxed 2/1-helical structure. According to a preliminary structural analysis, we found that not only the relaxed twofold helix, but also the fourfold helix, are favorable conformations for type II salts.[12] In this study, a more detailed structure for the chitosan/HI type II salt is reported.

2. Structural analysis

2.1. Crystal data

The X-ray diffraction pattern of the chitosan/HI Type II salt is shown in Figure 1. A total of 37 independent diffraction spots could be observed up to the 18th layer line, and these are indexed by a tetragonal unit cell with dimensions $a = b = 10.68(3)$ and c (fiber axis) = 40.77(13) Å. With the systematic absences on $00l$ reflections, $l \neq 4n$, the space group was assumed to be one of $P4_1$ and $P4_3$. However, the helical symmetry of this polymer was proposed to be 4/1 according to the relaxed potential energy surfaces of chitobiose[17] based on the MM3 calculation. So, we assumed $P4_1$ space group in this analysis.

The number of chitosan chains in the unit cell is 2 based on the observed density ($\rho_o = 1.81\,\mathrm{g\,cm^{-3}}$). Each chain has an octasaccharide in the c-repeat, the 4/1-helical symmetry coinciding with the crystallographic 4_1 symmetry. In addition, there should be sixteen halide ions in the unit cell (two per disaccharide repeat in each helix).

2.2. Molecular model building

Molecular models having 4/1-helical symmetry in the fiber period of 40.77 Å with a disaccharide as a helical asymmetric unit were generated by using WINLALS[18] program. The standard geometry of 2-amino-2-deoxy-D-glucose was obtained from the literature.[19] Atomic

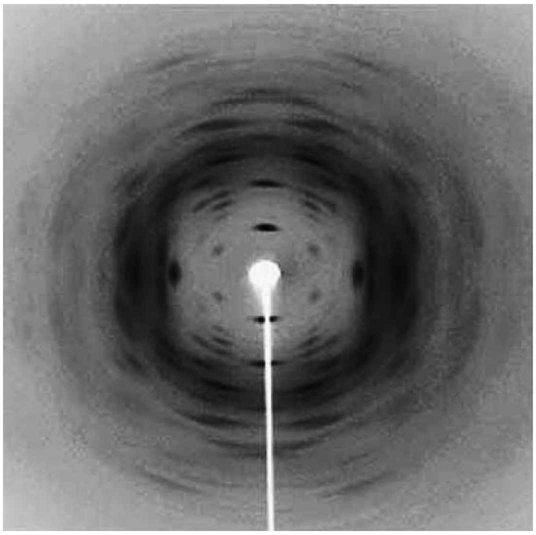

Figure 1. X-ray diffraction pattern of chitosan/HI type II salt recorded on an imaging plate (DIP-100S, MAC Science) using MoKα radiation of wavelength 0.7107 Å.

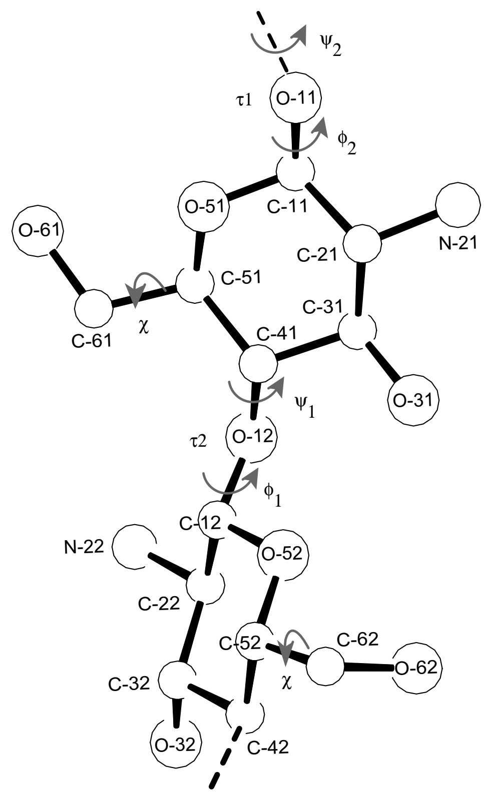

Figure 2. A disaccharide asymmetric unit of chitosan/HI type II salt together with atomic numberings, four conformational angles ϕ_1, ψ_1, ϕ_2, ψ_2 at the glycosidic linkage, two glycosidic bond angles τ_1, τ_2 and two O-6 orientation angles χ_1, χ_2.

numbering in an asymmetric unit is shown in Figure 2, together with four conformation angles ϕ_1(C-22–C-12–O-12–C-41), ψ_1(C-12–O-12–C-41–C-31), ϕ_2(C-21–C-11–O-11–C-42′), ψ_2(C-11–O-11–C-42′–C-32′) at the glycosidic linkages, and two glycosidic bond angles, τ_1(C-12–O-12–C-41), and τ_2(C-11–O-11–C-42′). Here, two torsion angles, χ_1(O-51–C-51–C-61–O-61) and χ_2(O-52–C-52–C-62–O-62) define the orientation of each O-6 oxygen atom. Many related compounds showed that this angle falls into one of the following three orientations:[20] *gauche–gauche* (*gg*, $\chi \approx -60°$), *gauche–trans* (*gt*, $\chi \approx 60°$), and *trans–gauche* (*tg*, $\chi \approx 180°$). As shown later, the *trans–gauche* (*tg*) orientation is rarely found in the oligomer[21] and was not considered. Therefore, structures with four combinations of the torsion angles χ_1 and χ_2, *gggg*, *gggt*, *gtgg*, and *gtgt*, were examined.

2.3. Packing of the chitosan chains and the iodide ions

Two chitosan chains are located at the corner and the center of the unit cell. Since the salt was prepared from tendon chitin in the solid state, the direction of the chains were preserved in an antiparallel fashion.[22] The corner molecule is oriented up, while the center molecule is oriented down. Although the two chains are independent, we assumed them to have the same conformation since there is no special reason to treat them otherwise.

There are four crystallographically independent iodide ions (I^-) in the unit cell. Their (u, v) positions were initially investigated by the F_o–F_c maps based on the $hk0$ reflections. To simplify the analysis, the orientation of the hydroxymethyl moiety was not considered at this stage by excluding the O-6 atom from the calculations. Starting from the difference map based on two polymer chains only in the unit cell, the locations of four iodide ions were obtained one by one (Fig. 3). Two of them were at about ($u = 0.0$, $v = 0.5$) and the other two around ($u = 0.25$, $v = 0.25$).

In the space group $P4_1$, one of the translation parameters of atoms along the *c*-axis is arbitrary. Therefore, the *z*-value of the first iodide ion ($w3$) was fixed at zero. The fractional coordinate w of the second iodide ion ($w4$) was searched in terms of the *R*-factors against all hkl reflections from $w = 0.0$ to 1.0 at 0.0125 (0.5 Å) interval. The structures at 38 positions out of 80, which had *R*-factors less than 0.42 were refined, which resulted in four local minima ($w4 = 0.10$, 0.39, 0.71, and 0.78). In each of them, the fractional coordinate w of the third ($w5$) and that of the fourth ($w6$) iodide ions were searched together. From these four, a total of 20 candidate positions where R fell in the minimum regions were selected. In the next step, the fractional coordinate w of two chains, $w1$ and $w2$, were investigated. From this stage, the noncovalent interatomic interaction was taken into account, and 5 out of 20 candidates were eliminated because of short contacts. Minimum regions in four *R*-maps showed lower R values than those in the other eleven *R*-maps. Five sets of $w1$, $w2$ were obtained from these four maps, two from one and one from each of the

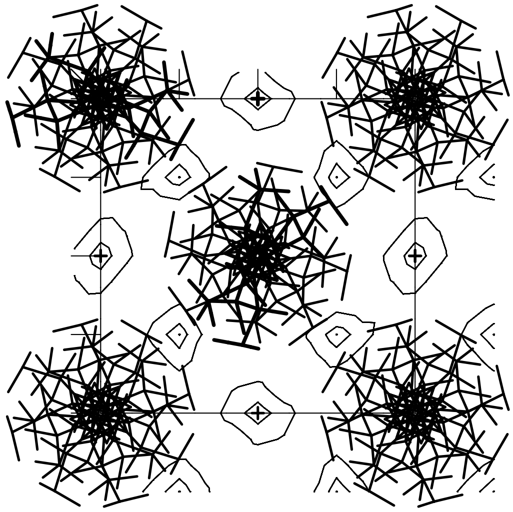

Figure 3. F_o–F_c map showing positions of the second and third iodide ions. F_c values were calculated for the polymer chains together with the first iodide ion ($I-1$) at $u = 0$, $v = 0.5$.

other three maps. All variable positional parameters (u, v, w) of these five candidates, together with the azimuthal angles (μ) of the two chains, were refined before subjecting the data to further analyses.

2.4. Determination of the O-6 orientation

According to the Cambridge Structural Database,[21] 109, 51, and 1 out of 161 examples in oligosaccharide crystals take *gg*, *gt*, and *tg* conformation, respectively. Based on this information, the *tg* orientation was not considered in this study. Since there are two pyranose residues in the asymmetric unit, the following four orientations: *gggg*, *gggt*, *gtgg*, and *gtgt*, were investigated. After including O-6 atoms, three candidate positions showed higher *R*-factors, while those of the other two were maintained or improved when compared with those before including O-6 atoms. The main difference between the latter two positions was w of the corner chain ($w1 \approx 0.31$ and $w1 \approx 0.25$). Therefore, eight models were further investigated.

In the next step, the chain conformational parameters (ϕ, ψ, τ) were also refined along with all variable positional parameters as well as the orientation of the O-6 atoms. *R*-factors (normal, weighted) of the eight models were in the range of (0.22–0.23, 0.24–0.26) and (0.24–0.27, 0.26–0.29) for excluded and included unobserved reflections, respectively. Since the X-ray intensity distribution in the diffraction pattern was mainly governed by the iodide ions, the difference of the polymer chains between eight models could not be clearly distinguished. Nevertheless, the model having $w1 \approx 0.31$ with *gtgg* or *gtgt* orientations showed low *R*-values. Therefore, we chose this model as the most plausible structure. Since the model with *gtgg* orientation showed better contacts between nonbonded atoms than the other one, this model was chosen as the most plausible model for the chitosan/HI type II salt.

3. Results and discussion

The final refined conformational and packing parameters of the *gtgg* model are shown in Table 1. The observed spacing (d_o), observed (F_o) and calculated (F_c) structure amplitudes are shown in Table 2. Fractional atomic coordinates of the disaccharide helical asymmetric unit of two chains and four iodide ions are shown in Table 3.

3.1. Molecular structures

The corner and center chains assume a fourfold helical symmetry with a repeating period of 40.77 Å and have a disaccharide as the helical asymmetric unit. Although there is a very strong hydrogen bond between O-3 and O-5 in the hydrated chitosan structure (2.60 Å)[4] to make a stable β-(1→4) glycosidic linkage, weak intramolecular hydrogen bonds at the glycosidic linkages (O-31···O-52: 2.96 Å, ∠ C-31–O-31···O52: 98.63°, O-32···O-51: 3.13 Å, ∠ C-32–O-32···O51: 97.48°) were observed in the chitosan/HI salt. The newly formed interactions with the iodide ions weaken the O-3···O-5 hydrogen bond at the glycosidic linkage of the hydrated chitosan. Because of this, an extended 2/1-helical conformation, which is strengthened by that hydrogen bond, is distorted, and consequently the 4/1-helical conformation seems to be adopted. The main chain conformation angles ϕ_1, ψ_1, ϕ_2, ψ_2 are 173.6°, 121.4°, 165.0°, and 126.9°, respectively. These deviate from the corresponding values (145.9°, 94.1°) for chitosan by about 19–33°. The glycosidic linkage angles, τ_1 and τ_2 are 116.7° and 116.5°. The O-6 atom of one residue prefers *gt*, while that of the other assumes a *gg* orientation ($\chi_1 = 52.1°$, $\chi_2 = -55.4°$). On the other hand, the orientation angles of the hydrated and anhydrous chitosan are reported to be 68.6° and 48.8°, respectively.[4,8]

Table 1. Final refined parameters of the most plausible model for chitosan/HI type II salt

Torsion angles and bond angles at the glycosidic linkage/°	
ϕ_1(C-22–C-12–O-12–C-41)	173.6
ψ_1(C-12–O-12–C-41–C-31)	121.4
ϕ_2(C-21–C-11–O-11–C-42′)	165.0
ψ_2(C-11–O-11–C-42′–C-32′)	126.9
τ_1(C-12–O-12–C-41)	116.7
τ_2(C-11–O-11–C-42′)	116.5
Orientation of the O-6 atoms/°	
χ_1(O-51–C-51–C-61–O-61)	52.1
χ_2(O-52–C-52–C-62–O-62)	−55.4
Packing parameters of chitosan chains and iodide ions	
Chitosan chains	
Corner chain μ_1/°	53.5
$w1$	0.354
Center chain μ_2/°	73.6
$w2$	0.339
Iodide ions	
I-1 ($u3, v3, w3$)	(−0.018, 0.503, 0.000)
I-2 ($u4, v4, w4$)	(−0.021, 0.541, 0.100)
I-3 ($u5, v5, w5$)	(0.263, 0.762, 0.827)
I-4 ($u6, v6, w6$)	(0.634, 0.758, 0.097)
Scale factor	1.7543
R-factors (R/R_w)	
Excluded unobserved reflections	0.23/0.24
Included unobserved reflections	0.25/0.26

3.2. Crystal structure

Viewing through the *ab*-plane, two independent chitosan chains are located at the corner and center of the unit cell. These two chains are arranged in an antiparallel fashion. Two independent iodide ions are packed between the corner chains, while the other two are between the corner and center chains (Fig. 4a). The packing of the iodide ions along the *c*-axis can be

Table 2. Observed spacings (d_o), observed (F_o)[a] and calculated (F_c) structure amplitudes for chitosan/HI type II salt

Spot no.	*h k l*	d_o	F_o	F_c
	1 0 0		(56)	64
	1 1 0		(67)	196
1	2 0 0	5.27	323	322
	1 2 0			
	2 1 0		(84)	123
2	2 2 0			
	3 0 0			
	1 3 0			
	3 1 0	3.68	787	656
3	2 3 0			
	3 2 0	3.01	241	161
4	4 0 0			
	1 4 0			
	4 1 0			
	3 3 0	2.65	521	422
	1 0 1		(56)	36
	1 1 1		(67)	77
	2 0 1		(80)	70
5	1 2 1			
	2 1 1	4.61	308	213
6	1 0 2	8.97	98	179
	1 0 3		(56)	77
7	1 1 3	6.61	82	153
	2 0 3		(80)	40
8	1 2 3			
	2 1 3	4.46	147	116
9	2 2 3	3.65	238	231
10	3 0 3			
	1 3 3			
	3 1 3	3.29	390	298
11	1 0 5	6.44	72	62
12	1 1 5	5.54	73	77
	2 0 5		(80)	137
13	1 2 5			
	2 1 5	4.12	142	226
14	2 2 5			
	3 0 5			
	1 3 5			
	3 1 5	3.29	265	240
15	1 0 6	5.67	120	139
16	1 1 6	4.97	117	224
	2 0 6		(80)	55
17	1 2 6			
	2 1 6	3.89	318	206
18	1 0 7	5.10	101	135
19	1 0 8	4.52	102	116
20	1 1 8	4.18	213	282
21	2 0 8			
	1 2 8			
	2 1 8	3.64	295	327
22	2 2 8			
	3 0 8	3.03	260	314
23	1 3 8			
	3 1 8	2.82	240	269
24	1 0 9	4.17	68	108
	1 1 9		(67)	39
25	2 0 9			
	1 2 9			
	2 1 9	3.31	181	191
26	1 0 10			
	1 1 10	3.81	285	339
27	1 0 11	3.52	132	249
28	1 1 11	3.31	139	90
	2 0 11		(80)	31
	1 2 11			
	2 1 11		(84)	55
29	2 2 11			
	3 0 11			
	1 3 11			
	3 1 11	2.60	331	314
30	1 0 13	2.99	163	151
31	1 1 13	2.88	193	127
32	2 0 13			
	1 2 13			
	2 1 13	2.65	196	224
33	1 0 14			
	1 1 14			
	2 0 14	2.72	106	193
34	1 0 15			
	1 1 15	2.65	200	178
	1 0 16		(51)	39
	1 1 16		(64)	82
35	2 0 16			
	1 2 16			
	2 1 16	2.30	223	191
36	1 0 18			
	1 1 18	2.17	176	185
37	2 0 18			
	1 2 18			
	2 1 18	2.06	263	262

[a] Reflections with F_o values in parentheses are those for unobserved reflections. These values are two-thirds of the observational threshold.

explained as follows. One iodide ion packed between the corner chains is at the bottom of the unit cell, while the second iodide ion, which packed in a similar (x, y) position is about 4 Å apart. The third and the fourth iodide ions packed at a similar (x, y) position are about 20 Å apart (Fig. 4b).

3.3. Nonbonded interactions among polymer chains and iodide ions

Two independent chains are linked to each other by two N-21···O-61 hydrogen bonds (2.64 and 2.68 Å). The iodide ions are stabilized in the structure by forming hydrogen bonds to N-21, N-22, O-32, O-61, O-62 atoms of the polymer chains (Table 4). The O···I^- and N···I^- distances found in this study are 3.22–3.58 and 3.51–3.70 Å, respectively. The first independent iodide ion (I-1) forms hydrogen bonds with N-22 of the corner chain. The second iodide ions (I-2) interact with N-21 and O-61 of the center chains. The O-32 atoms of both chains donate hydrogen to the third iodide ions (I-3). The interaction between these O-32 and I-3 seems to cause the main chain to be in the 4/1-helical conformation. The fourth iodide ions form hydrogen bonds to the N-21, and O-61 of the corner chains and N-21, and O-62 of the center chains.

Table 3. Fractional atomic coordinates of disaccharide helical asymmetric unit of two chains[a] and four iodide ions

Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*
Corner chain				H-42	0.032	0.077	0.406	C-42	0.560	0.482	0.282
O-11	0.018	−0.095	0.630	H-52	−0.120	−0.112	0.439	H-12	0.603	0.379	0.210
C-11	0.017	−0.013	0.604	H-62a	0.039	−0.203	0.405	H-22	0.697	0.618	0.238
O-51	−0.107	−0.010	0.592	H-62b	0.055	−0.237	0.444	H-32	0.717	0.377	0.269
C-51	−0.119	0.074	0.564	*Center chain*				H-42	0.542	0.572	0.287
C-61	−0.257	0.078	0.555	O-11	0.413	0.457	0.062	H-52	0.483	0.336	0.254
O-61	−0.333	0.106	0.583	C-11	0.479	0.506	0.088	H-62a	0.315	0.409	0.287
C-21	0.108	−0.061	0.578	O-51	0.557	0.408	0.101	H-62b	0.278	0.401	0.249
N-21	0.239	−0.061	0.592	C-51	0.631	0.449	0.129	*Iodide ions*			
C-31	0.102	0.018	0.547	C-61	0.717	0.342	0.137	I-1	−0.018	0.503	0.000
O-31	0.178	−0.036	0.522	O-61	0.786	0.298	0.110	I-2	−0.021	0.541	0.100
C-41	−0.033	0.027	0.535	C-21	0.386	0.550	0.114	I-3	0.263	0.762	0.827
H-11	0.015	0.077	0.611	N-21	0.308	0.654	0.100	I-4	0.634	0.758	0.097
H-21	0.087	−0.150	0.573	C-31	0.453	0.592	0.145				
H-31	0.134	0.104	0.552	O-31	0.364	0.620	0.170				
H-41	−0.061	−0.056	0.527	C-41	0.542	0.490	0.157				
H-51	−0.093	0.160	0.571	H-11	0.553	0.558	0.081				
H-61a	−0.271	0.144	0.538	H-21	0.327	0.479	0.120				
H-61b	−0.282	−0.006	0.546	H-31	0.502	0.670	0.140				
O-12	−0.042	0.115	0.509	H-41	0.492	0.417	0.165				
C-12	−0.089	0.070	0.480	H-51	0.684	0.522	0.122				
O-52	0.001	−0.017	0.467	H-61a	0.778	0.370	0.154				
C-52	−0.038	−0.070	0.436	H-61b	0.665	0.271	0.146				
C-62	0.058	−0.168	0.427	O-12	0.618	0.536	0.183				
O-62	0.182	−0.118	0.427	C-12	0.610	0.472	0.213				
C-22	−0.105	0.181	0.456	O-52	0.486	0.491	0.226				
N-22	−0.201	0.269	0.470	C-52	0.467	0.428	0.256				
C-32	−0.143	0.136	0.422	C-62	0.331	0.445	0.265				
O-32	−0.145	0.239	0.400	O-62	0.296	0.574	0.266				
C-42	−0.051	0.037	0.410	C-22	0.708	0.525	0.236				
H-12	−0.159	0.009	0.483	N-22	0.836	0.501	0.223				
H-22	−0.025	0.228	0.455	C-32	0.694	0.468	0.270				
H-32	−0.229	0.099	0.423	O-32	0.778	0.528	0.292				

[a] Corner and center chains have the same conformation except orientation of O-6 atoms.

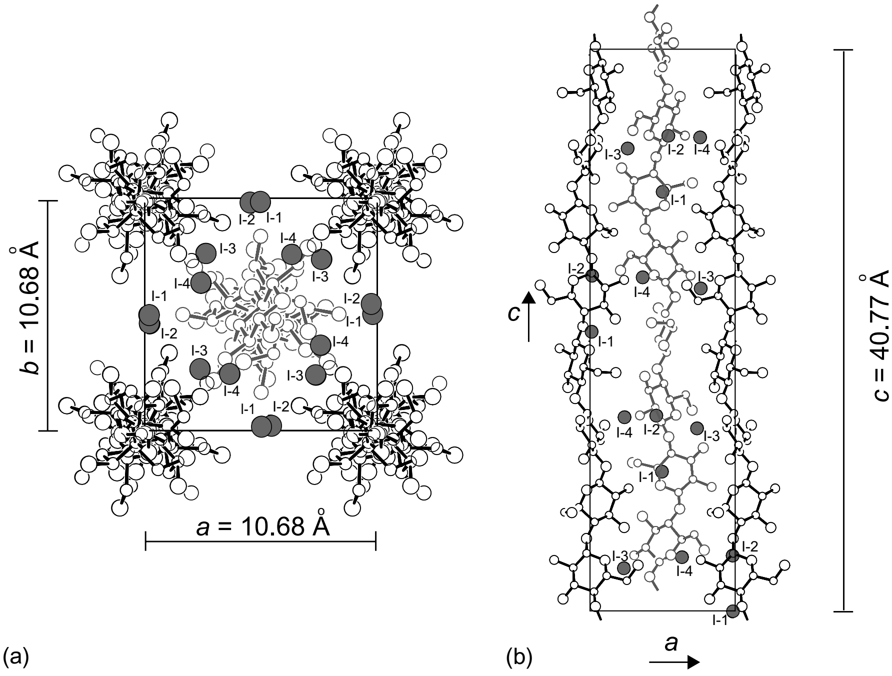

Figure 4. Crystal packing of chitosan/HI type II salt (a) projected along the *c*-axis, and (b) projection along the *b*-axis. Filled circles represent the iodide ions.

Table 4. Attractive interactions among chitosan chains and iodide ions of the final models

Atom X	Atom Y	X—Y/Å
N-21	O-61[a] (i)[b]	2.64
N-21	I-4 (ii)[b]	3.51
N-22	I-1 (iii)[b]	3.60
O-32	I-3 (iv)[b]	3.22
O-61	I-4 (iii)[b]	3.58
N-21[a]	O-61 (iv)[b]	2.68
N-21[a]	I-2	3.70
N-21[a]	I-4	3.68
O-32[a]	I-3 (v)[b]	3.44
O-61[a]	I-2 (vi)[b]	3.34
O-62[a]	I-4 (vii)[b]	3.43

[a] Denotes the atom of the center chain.

[b] Symmetry codes: (i) $-x$, $-1-y$, $-0.5+z$; (ii) $1-x$, $1-y$, $0.5+z$; (iii) $-x$, $1-y$, $0.5+z$; (iv) $-x$, $1-y$, $-0.5+z$; (v) $1-x$, $1-y$, $-0.5+z$; (vi) $1+x$, y, z; (vii) $1-y$, x, $0.25+z$.

3.4. Crystal transformation from type II complexes to anhydrous chitosan

The anhydrous crystalline form of chitosan is usually obtained from a high-temperature treatment,[5] which causes a little loss in orientation and decomposition, particularly on the surface of a specimen. On the other hand, crystal transformation from type II complexes to this crystalline form occurs under mild conditions.[6,7] Therefore, the orientation and crystallinity are well preserved. To understand the transformation mechanism, the structure of anhydrous chitosan and type II complexes should be clarified. A detailed structure of anhydrous chitosan is already reported,[8] and we have determined structure of type II complex in this study. The transformation mechanism is now underway.

4. Conclusions

The structure of the chitosan/HI type II salt was investigated by the X-ray diffraction technique. Different from other type II salts, a tetragonal unit cell $a = b = 10.68(3)$, c (fiber axis) $= 40.77(13)$ Å could index the observed 37 spots. Two polymer chains (16 sugar units) and 16 iodide ions (I^-) are in the unit cell according to space group $P4_1$. Viewed in the *ab*-plane, two helices are positioned at the corner and center of the unit cell. Two out of four of the iodide ions in the crystal asymmetric unit are present between the corner chains, and the other two are situated between the corner and center chains. The O-3···O-5 intramolecular hydrogen bonds that strengthen the typical extended 2/1-helical conformation are weakened by the interaction with iodide ions, giving rise to the new relaxed 4/1-helical conformation. The helical asymmetric unit consists of two pyranose rings. The orientation of O-6 in the first residue is *gt*, while that in the second residue is *gg*. The corner chains are linked to the adjacent center chains by N-21···O-61 hydrogen bonds. Four independent iodide ions form hydrogen bonds with N-21, N-22, O-32, O-61, and O-62 atoms of the polymer chains.

5. Experimental section

5.1. Materials

The tendons of *Chionoecetes opilio* O. Fabricius crab was used to prepare tendon chitosan. The specimens were immersed in 2 M HCl solution at room temperature overnight in order to remove inorganic compounds. Then N-deacetylation was performed twice with 50% sodium hydroxide solution at 110 °C for 2 h under a nitrogen atmosphere. The degree of N-acetylation of the specimen obtained by the above procedure was known to be almost 0% by the colloidal titration and the viscosity-average degree of polymerization at least 10,800.[16] The type II salt was obtained by immersing tendon chitosan into 6.0 M HI at 4 °C for 24 h, then washing in 75% 2-propanol and pure 2-propanol. The specimen was dried in air before the X-ray measurement. The density of the specimen was measured by a flotation method using a mixture of 1,2-dibromoethane and 1,2-dichloroethane.

5.2. X-ray diffraction measurement

The X-ray diffraction pattern of the chitosan/HI salt was recorded on an Imaging Plate (DIP-100S, MAC Science) mounted on a Rigaku ultraX18 rotating anode X-ray generator, operated in normal focus mode at 50 kV and 250 mA. The specimen was exposed to a graphite-monochromatized MoKα radiation ($\lambda = 0.7107$ Å) collimated with a pinhole of diameter 0.5 mm for 1 h. The camera-to-sample distance was calibrated using NaCl powder.

5.3. X-ray intensity measurement

The in-house data processing software was used to obtain the X-ray intensity (I_o) for each diffraction spot and to determine the unit cell parameters.[4,23,24] The measured intensities (I_o) were corrected for the Lorentz and polarization factors. To avoid the absorption effect, the molybdenum (Mo) target was used in this study instead of copper (Cu). The Lorentz factor, L, is given by the following expression when there is no fiber tilting to the normal to the X-ray beam:[25]

$$\frac{1}{L} = 2\pi \sin\theta \sqrt{\cos^2\theta - \cos^2\sigma}$$

in which $\tan\sigma = \zeta/\xi$. The polarization factor, p, is given by the following expression when the primary beam is partially polarized by a monochromator crystal:[26]

$$p = \frac{(\cos^2 2\theta_{\mathrm{M}} \cos^2\rho + \sin^2\rho)\cos^2 2\theta + \cos^2 2\theta_{\mathrm{M}} \sin^2\rho + \cos^2\rho}{1 + \cos^2 2\theta_{\mathrm{M}}}.$$

Here, θ is the Bragg angle of the reflection, θ_{M} is the Bragg angle of the 002 reflection plane of the carbon graphite monochromator ($\theta_{\mathrm{M}} = 6.09°$). (ξ, ζ) are the cylindrical polar coordinates in reciprocal space, and the $\cos\rho = \zeta/\sin 2\theta$ when both the diffraction plane of the monochromator and the fiber axis are horizontal setting. Then, the observed structure factor, F_{o}, will be obtained by

$$F_{\mathrm{o}} = \sqrt{\frac{I_0}{Lp}}.$$

5.4. Linked-atom least-square analysis

At each stage of establishing molecular and packing models and refinement, the quantity Ω was minimized.

$$\Omega = \sum_m w_m(|F_m^{\mathrm{o}}| - |F_m^{\mathrm{c}}|)^2 + \sum_i k_i(d_i - d_i^{\mathrm{o}})^2 + \sum_j e_j(p_j - p_j^{\mathrm{o}})^2 + \sum_q \lambda_q G_q.$$

The first term represents the differences between the observed (F_{o}) and calculated (F_{c}) structure amplitudes. The weight of the reflection, w, was fixed to 1.0 for all observed reflections, −1.0 for the unobserved ones of which $|F_{\mathrm{c}}| > |F_{\mathrm{unobs}}|$. For those which $|F_{\mathrm{c}}| < |F_{\mathrm{unobs}}|$, the w was set as 0. The unobserved reflections with longer spacings than that of the outermost reflection on the same layer line were also included in the structure analysis and assumed to have two-thirds of the intensity of the observational threshold. The second term ensures the optimization of noncovalent interatomic interactions. The third term is used to restrain certain parameters or quantities, such as torsion angles, bond angles or interatomic distances to their expected or standard values. The last term is used to impose the exact relationships and constraints that we have chosen. The constraints are usually applied for preserving helix continuity. The agreement between the observed and calculated structure amplitudes is described in terms of the crystallographic R, which are defined by

$$R \text{ (normal)} = \frac{\sum ||F_{\mathrm{o}}| - |F_{\mathrm{c}}||}{\sum |F_{\mathrm{o}}|},$$

$$R_{\mathrm{w}} \text{ (quadratic weighted)} = \left[\frac{\sum w(|F_{\mathrm{o}}| - |F_{\mathrm{c}}|)^2}{\sum wF_{\mathrm{o}}^2}\right]^{1/2}.$$

Atomic scattering factors for calculating structure factors were obtained by using the method and values given in the literature.[27] The attenuation factor was fixed as 8.00 through out the refinement. Computations were carried out on PC using WINLALS[18] version 1.105.

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References

1. Li, Q.; Dunn, E. T.; Grandmaison, E. W.; Goosen, M. F. A. Applications and properties of chitosan. In *Applications of Chitin and Chitosan*; Goosen, M. F. A., Ed.; Technomic Publishing: Lancaster, PA, 1997; pp 3–21.
2. Muzzarelli, R. A. A. *Natural Chelating Polymers: Algenic Acid, Chitin and Chitosan*; Pergamon: Oxford, 1973; pp 145–176.
3. Clark, G. L.; Smith, A. F. *J. Phys. Chem.* **1936**, *40*, 863–879.
4. Okuyama, K.; Noguchi, K.; Miyazawa, T.; Yui, T.; Ogawa, K. *Macromolecules* **1997**, *30*, 5849–5855.
5. Ogawa, K.; Hirano, S.; Miyanishi, T.; Yui, T.; Watanabe, T. *Macromolecules* **1984**, *17*, 973–975.
6. Kawada, J.; Abe, Y.; Yui, T.; Okuyama, K.; Ogawa, K. *J. Carbohydr. Chem.* **1999**, *18*, 559–571.
7. Kawada, J.; Yui, T.; Okuyama, K.; Ogawa, K. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 2542–2547.
8. Okuyama, K.; Noguchi, K.; Hanafusa, Y.; Osawa, K.; Ogawa, K. *Int. J. Biol. Macromol.* **1999**, *26*, 285–293.
9. Okuyama, K.; Noguchi, K.; Kanenari, M.; Egawa, T.; Osawa, K.; Ogawa, K. *Carbohydr. Polym.* **2000**, *41*, 237–247.
10. Ogawa, K.; Inukai, S. *Carbohydr. Res.* **1987**, *160*, 425–433.
11. Ogawa, K.; Nakata, K.; Yamamoto, A.; Nitta, Y. *Chem. Mater.* **1996**, *8*, 2349–2351.
12. Lertworasirikul, A.; Tsue, S.; Noguchi, K.; Okuyama, K.; Ogawa, K. *Carbohydr. Res.* **2003**, *338*, 1229–1233.
13. Okuyama, K.; Osawa, K.; Hanafusa, Y.; Noguchi, K.; Ogawa, K. J. *Carbohydr. Chem.* **2000**, *19*, 789–794.
14. Kawahara, M.; Yui, T.; Oka, K.; Zugenmaier, P.; Suzuki, S.; Kitamura, S.; Okuyama, K.; Ogawa, K. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 1545–1550.
15. Kawada, J.; Yui, T.; Abe, Y.; Ogawa, K. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 700–704.
16. Ogawa, K. *Agric. Biol. Chem.* **1991**, *55*, 2375–2379.
17. Yui, T.; Kobayashi, H.; Kitamura, S.; Imada, K. *Biopolymers* **1994**, *34*, 203–208.
18. Okada, K.; Noguchi, K.; Okuyama, K.; Arnott, S. *Comput. Biol. Chem.* **2003**, *27*, 265–285.
19. Arnott, S.; Scott, W. E. *J. Chem. Soc., Perkin Trans. 2* **1972**, 324–335.
20. Sundaralingam, M. *Biopolymers* **1968**, *6*, 189–213.
21. Allen, F. H.; Bellard, S.; Brice, M. D.; Cartwright, B. A.; Doubleday, A.; Higgs, H.; Hummelink, T.; Hummerlink-Peters, B. G.; Kennard, O.; Motherwell, W. D. S.; Rodgers, J. R.; Watson, D. G. *Acta Crystallogr.* **1979**, *B35*, 2331–2339.
22. Minke, R.; Blackwell, J. *J. Mol. Biol.* **1978**, *120*, 167–181.
23. Obata, Y.; Okuyama, K. *Kobunshi Ronbunshu* **1994**, *51*, 371–378; *Chem. Abstr.* **1994**, *121*, 85552.

24. Okuyama, K.; Obata, Y.; Noguchi, K.; Kusaba, T.; Ito, Y.; Ohno, S. *Biopolymers* **1996**, *38*, 557–566.
25. Millane, R. P.; Arnott, S. *J. Macromol. Sci. Phys.* **1985**, *B24*, 193–227.
26. Azaroff, L. V. *Acta Crystallogr.* **1955**, *8*, 701–704.
27. *International Tables for Crystallography*; Ibers, J. A., Hamilton, W. C., Eds.; Kynoch: Birmingham, UK, 1974; Vol. 4, pp 71–147.