726 Chem. Mater. **1993,** *5,* 726-728

X-ray Study of Chitosan-Transition Metal Complexes

Kozo Ogawa* and Kunio Oka

Research Institute for Advanced Science and Technology, University of Osaka Prefecture,
1-2 Gakuen-cho, Sakai, Osaka 593, Japan

Toshifumi Yui

Department of Industrial Chemistry, Miyazaki University, Miyazaki 889-21, Japan

Received January 19, 1993. Revised Manuscript Received February 17, 1993

Clear fiber diffraction patterns of nine chitosan-transition metal salt [$CdSO_4$, $CdCl_2$, $Cd(NO_3)_2$, $Cd(CH_3COO)_2$, $ZnSO_4$, $ZnCl_2$, $Zn(CH_3COO)_2$, $CuSO_4$, and $CuCl_2$] complexes were obtained by immersing a tendon chitosan, prepared by a solid-state N-deacetylation of a crab tendon chitin, in respective metal salt solutions. The extended 2-fold helix of the chitosan molecule was retained even with the complex formation. The unit cells of all the salt complexes studied were orthorhombic though the cupric salt complexes showed some unindexed reflections. Their lattice parameters and number of water molecules in the cell depended on the counteranions of the metal salt and not on the metal ion. The ratio of glucosamine residue to metal salt was 2:1. The space group $P2_12_12_1$ was suggested for all the complex crystals except the $CuCl_2$ complex.

Introduction

Chitosan, poly(*N*-acetyl-D-glucosamine) and the N-deacetylation product of chitin, is a promising bioresource for industrial purposes and is under intensive investigation for this application. It has a regular distribution of aliphatic primary amino groups on the chain and is a first-rank chelating polymer of transition metals.[1] Muzzarelli and co-workers found that chitosan collects metal ions selectively, that is, it does not take up alkali- and alkali-earth metal ions but it collects transition and post-transition metal ions from aqueous solution.[1] Since the dissociation of a chitosan-metal complex occurs easily by lowering the pH chitosan has been applied to the recovery of useful transition metals from waste. From a scientific point of view two coordination modes have been proposed for chitosan-metal complexes and they are under debate. One is the so-called "bridge model" where a metal ion such as cupric ion is coordinated with four nitrogen atoms of intra- and inter-chitosan chains.[2,3] On the contrary, based on an X-ray study of the chitosan complexes with several metal salts we proposed another coordination mode called the "pendant model" in which a metal ion is attached to an amino group of the chitosan chain like a pendant.[4] This model was supported by Domard[5] from his study on the solution behavior of a chitosan-cupric complex. At present, both models have no clear experimental evidence. The deficiency in our proposal came from the poor quality of the X-ray fiber diagrams of the chitosan metal complexes which were prepared by using a stretched chitosan film.[4] However, we have now succeeded in obtaining high-quality fiber patterns of the complexes, shown in this preliminary report, using tendon chitosan[6,7] prepared by a solid state deacetylation of a crab tendon chitin.

Table I. Optimum Conditions for Making Crystals of Chitosan-Metal Complexes

salt	concn (M)	pH of salt soln	reaction time (h)	wt % metal ion in the product
$CdSO_4$	1	4.11	4	15.5
$CdCl_2$	1	4.54	5	18.3
$Cd(NO_3)_2$	1	3.45	5	11.9
$Cd(ClO_4)_2$[a]	1	4.25[b]	18	
$Cd(CH_3COO)_2$	0.4	6.77	1	13.8
$ZnSO_4$	1	4.80	8	8.8
$ZnCl_2$	0.4	5.65	1	9.7
$Zn(NO_3)_2$[a]	1	4.59	20	
$Zn(ClO_4)_2$[a]	0.8	4.54	48	
$Zn(CH_3COO)_2$	0.4	6.18	1	6.8
$CuSO_4$	1	3.38	6	6.3
$CuCl_2$	0.4	3.31	22	11.7
$Cu(CH_3COO)_2$[a]	0.1	5.50	30	

[a] These salts always gave mixtures of chitosan and respective complexes. [b] The pH was maintained by adding 0.1 M NaOH solution.

Experimental Section

Materials. Tendon chitosan was prepared from the chitin of a crab tendon, *Chionecetes opilio* O. Fabricus, by N-deacetylation with 67% sodium hydroxide solution at 110 °C for 2 h under nitrogen atmosphere. This deacetylation procedure was repeated twice. The degree of N-acetylation of the resultant tendon chitosan was found to be 0% by the measurement of a colloidal titration, and the viscosity average degree of polymerization was 10 800.[8] To improve its accessibility to a metal salt solution, the chitosan was pretreated by immersing in methyl alcohol and water. Approximately 4 mg of the tendon chitosan, which is equivalent to 2.5×10^{-5} M in the glucosamine residue, was soaked with each aqueous solution (100 mL) of following metal salts at room temperature: $CdSO_4$, $CdCl_2$, $Cd(NO_3)_2$, $Cd(ClO_4)_2$, $Cd(CH_3COO)_2$, $ZnSO_4$, $ZnCl_2$, $Zn(NO_3)_2$, $Zn(ClO_4)_2$, $Zn(CH_3COO)_2$, $CuSO_4$, $CuCl_2$ and $Cu(CH_3COO)_2$, followed by washing with water and drying in air. To find the optimum conditions for getting the best fiber pattern of each chitosan-metal complex, the metal salt concentration and immersion period of the chitosan in the

---

[1] Muzzarelli, R. A. A. *Chitin*; Pergamon Press: Oxford, 1977.

[2] Yaku, F.; Muraki, E.; Tsuchiya, K.; Shibata, Y.; Koshijima, T. *Cellulose Chem. Technol.* 1977, *11,* 421.

[3] Schlick, S. *Macromolecules* **1986,** *19,* 192.

[4] Ogawa, K.; Oka, K.; Miyanishi, T.; Hirano, S. In *Chitin, Chitosan and Related Enzymes*; Zikakis, J. P., Ed.; Academic Press: Orlando, 1984; pp 327-345.

[5] Domard, A. *Int. J. Biol. Macromol.* 1987, *9,* 98.

[6] Clark, G. L.; Smith, A. F. *J. Phys. Chem.* **1937,** *40,* 863.

[7] Ogawa, K.; Hirano, S.; Miyanishi, T.; Yui, T.; Watanabe, T. *Macromolecules* **1984,** *17,* 973.

[8] Ogawa, K. *Agric. Biol. Chem., Tokyo* **1991,** *55,* 2375.

0897-4756/93/2805-0726$04.00/0

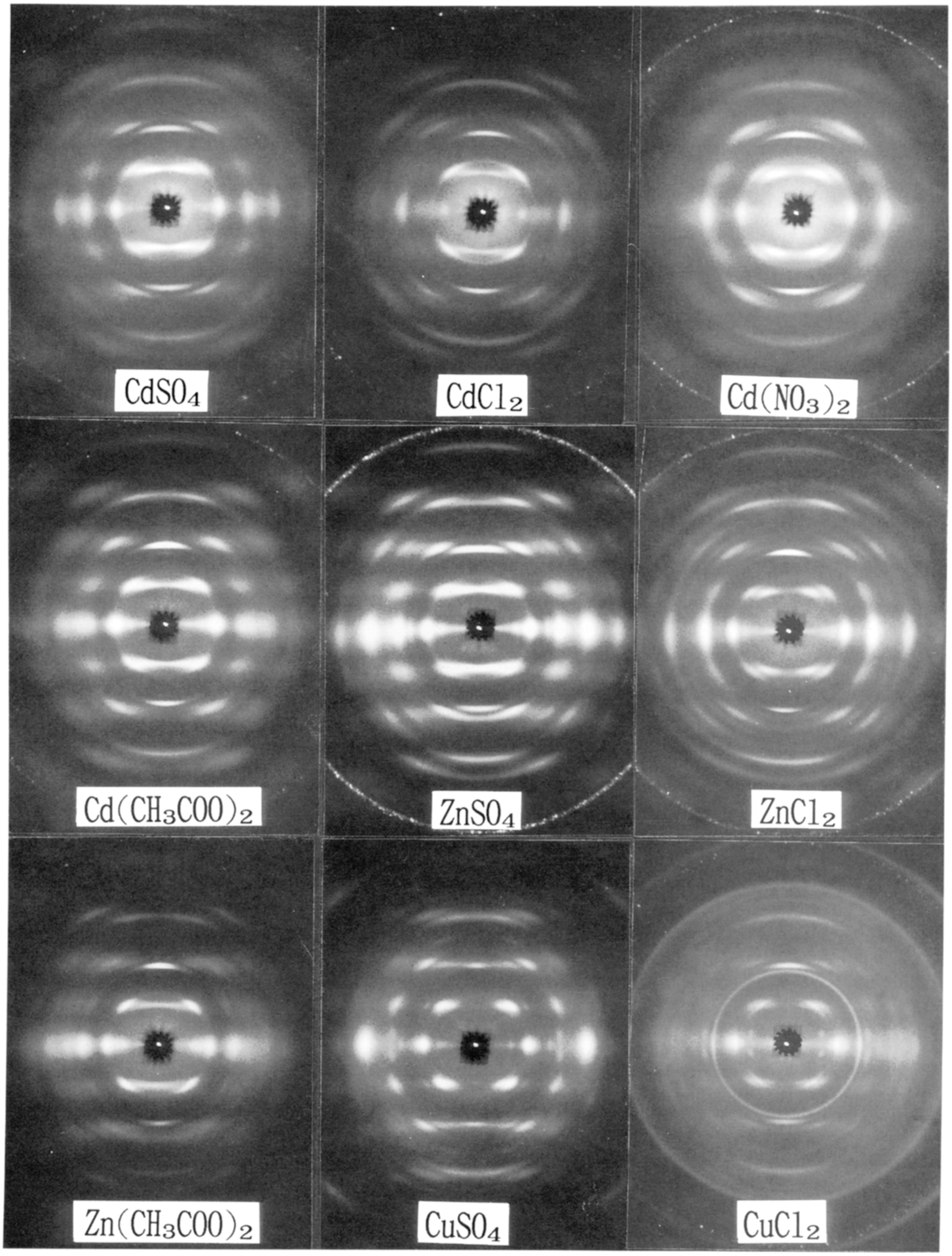

Figure 1. Fiber diffraction patterns of various chitosan–transition metal salt complexes. The dotted circle is the diffraction of NaF powder, which is a standard for measuring the distance between the sample and X-ray film.

salt solution were varied from 0.01 to 1 M and 1 h to 2 days, respectively, where the quantity of metal ion was large compared to that of the glucosamine residue of the chitosan (2.5×10^{-5} M). The pH of 1 M $Cd(ClO_4)_2$ aqueous solution was 0.92. At such low pH, chitosan is dissolved in the solution, and no fiber pattern of the complex can be obtained. Thus, before soaking the tendon chitosan, the pH of the $Cd(ClO_4)_2$ solution increased to 4.25 by addition of 4 mL of 0.1 M aqueous NaOH to 100 mL of the salt solution.

Methods. The density of each chitosan–metal complex was measured by a flotation method in a carbon tetrachloride-ethylene dibromide solution at 20 °C except those of $ZnCl_2$ and $Zn(CH_3COO)_2$ complexes because their observed density values were less than that of carbon tetrachloride (1.60 at 20 °C). They were measured in a carbon tetrachloride–*m*-xylene mixture. To confirm complex formation each sample was dissolved in a 0.1 M HCl solution and the metal ion concentration was measured by an atomic absorption spectrophotometer. The X-ray dif-

Table II. Unit-Cell Parameters of Chitosan–Transition Metal Complexes

metal salt	parameters				no. of			density	
	a (Å)	b (Å)	c (Å)[a]	vol (Å^3)	R[b]	M[c]	H_2O	calc	obs
$CdSO_4$	20.24	25.50	10.36	5,347	16	8	88(11)	1.80	1.78
$ZnSO_4$	21.00	25.65	10.36	5,580	16	8	88(11)	1.62	1.62
$CuSO_4$	20.46	25.65	10.36	5,437	16	8	88(11)	1.66	1.64
$CdCl_2$	23.96	17.55	10.35	4,352	16	8	40(5)	1.82	1.77
$ZnCl_2$	24.00	17.56	10.36	4,366	16	8	40(5)	1.67	1.56
$CuCl_2$	24.00	17.55	10.36	4,364	16	8	40(5)	1.66	1.63
$Cd(AcO)_2$	22.76	20.77	10.36	4,897	16	8	32(4)	1.69	1.65
$Zn(AcO)_2$	22.36	20.84	10.36	4,828	16	8	32(4)	1.59	1.55
$Cd(NO_3)_2$	20.82	20.48	10.37	4,422	16	8	24(3)	1.84	1.81

[a] Fiber axis. [b] R: glucosamine residue. [c] M: metal salt. ( ): number of water molecules per counter ion of salt.

fraction patterns were recorded by using a flat-film camera at 100% relative humidity in a helium atmosphere with a Rigaku Geigerflex X-ray diffractometer employing Ni-filtered Cu Kα radiation generated at 40 kV and 15 mA. When the X-ray measurements were done under vacuum all the complexes showed diffuse fiber patterns indicating the presence of water molecules in the crystals.

Results and Discussion

The optimum salt concentration and immersion period for getting a clear X-ray fiber pattern of each complex depended on the kind of salt (Table I). Lower salt concentrations or shorter immersion periods gave X-ray patterns characteristic of a mixture of unreacted chitosan and complex. In the case of cupric salt complexes, higher concentrations or longer periods caused a lowering of the degree of crystallinity. Among 13 metal salts complexes, the $Cd(ClO_4)_2$, $Zn(NO_3)_2$, $Zn(ClO_4)_2$, and $Cu(CH_3COO)_2$ complexes were discarded since they always gave fiber patterns of indicative of mixtures of chitosan and the respective complexes. The remaining nine chitosan-metal salt complexes gave clear fiber patterns, although $CuCl_2$ solution always gave X-ray patterns having rings of an unknown compound (Figure 1). The weight percentage of the metal ion and the observed density of each complex are shown in Tables I and II, respectively.

All the reflections observed on each pattern of the complexes of cadmium and zinc salts could be indexed with orthorhombic unit cells (Tables II and IIIa–g; Table III is included as supplementary material). The crystals of the cupric complexes, the most popular among the chitosan–transition metal complexes, are rather complicated, however. The $CuSO_4$ complex showed a very weak (001) and five unindexed reflections beside the layer lines (Figure 1 and Table IIIh). Two unindexed reflections between zero- and first-layer lines are also observed in the $CuCl_2$ complex (Figure 1 and Table IIIi). These may occur from imperfections in these crystals. However, the principal unit cell of each cupric complex was determined to correspond to the orthorhombic system, as shown in Tables II, IIIh, and IIIi.

The layer line spacing (approximately 10.36 Å) of all the complexes is similar to that of tendon chitosan,[6] indicating that the extended 2-fold helical conformation of the chitosan molecule is retained even after complex formation. The unit cell volume coupled with the observed density of each complex indicates that the relative number of glucosamine residues (16) and metal salts (8) in unit cell are the same in all of the complex crystals (Table II); i.e., 2:1. On the other hand, the number of water molecules present depended on the counter (acid) ion of the metal salt and not on the metal ion. The axial lengths of each complex depended on the counter ion of the metal salt as well. These results suggest that size of the unit cell is affected by the number of water molecule hydrating mainly to a counter ion. The retainment of the extended 2-fold helix of chitosan molecule by the complex formation indicates that there are eight chains of chitosan–metal salt complex in each unit cell. In addition, a space group $P2_12_12_1$ was suggested at each unit cell of all the complexes except the $CuCl_2$ complex, assuming that the presence of the very weak (001) reflection and/or of unindexed reflections in the $CuSO_4$ complex (Table IIIh) are due to an imperfection in the crystal. The crystal of the $CuCl_2$ complex could not have a $P2_12_12_1$ space group because of the presence of the very strong reflection indexed (300) (Table IIIi), however. These observations seem to support our proposed "pendant model" for the coordination mode of a chitosan–transition metal complex where a metal salt is coordinated with an amino group of a dimer residue of chitosan.[4] Further analysis is underway.

So far, three crystalline polymorphs have been reported for chitosan: tendon,[6,7] L-2,[9] and annealed[7] polymorphs. The former two are hydrated and the last, anhydrous. We reported[4] that when the stretched chitosan films showing a tendon polymorph but of low crystallinity[7] were soaked in the transition-metal salt solutions, the resultant films gave diffused fiber patterns of the complexes. In contrast, the present chitosan prepared from the tendon chitin showed a tendon polymorph of high crystallinity[8] and gave clear fiber patterns of the metal salt complexes when it was soaked in the salt solutions. On the other hand, the annealed chitosan film which was prepared by heating a chitosan film in water at a high temperature gave a typical annealed polymorph of high crystallinity[7] and did not make any complex with the metal salts, i.e., there was observed no change in the fiber pattern even when it was soaked in the metal salt solutions. These observations suggest that in order to get a chitosan–metal salt complex of high crystallinity a chitosan sample with well-crystallized hydrated polymorph must be used.

Supplementary Material Available: Listing of the observed spacings and intensities for nine chitosan-transition metal complex crystals (Table IIIa–i, 9 pages). Ordering information is given on any current masthead page.

---

(9) Sakurai, K.; Shibano, T.; Kimura, K.; Takahashi, T. *Sen-i Gakkaishi, Tokyo* 1985, *41*, T361.