Guest Selectivity in Complexation of β-Chitin

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ABSTRACT: Guest selectivity in complexation of β-chitin with small molecules was studied. Since amines are strong agents for β-chitin complexation, selectivity for various amines was studied first. Of three isomers of phenylenediamine, the *para* isomer formed a complex, while *ortho* and *meta* isomers did not. The selectivity for linear aliphatic amines was examined for equimolar mixtures of various combinations, i.e., primary−secondary−tertiary amines of C_6 main chain; C_5 diamine and C_6 monoamine; and C_6 monoamine and C_8 monoamine. The results showed a clear selectivity for primary amine over secondary and tertiary, diamine over monoamine, and longer alkyl chain over a shorter one. On the basis of such selectivity, complexation by guest incorporation from solution was established. This procedure allowed β-chitin complexation with high melting point compounds, to which pure liquid treatment was not applicable. By the solution treatment, complexes with acrylamide, *p*-aminobenzoic acid, and D-glucose were successfully prepared. In this method, even low-solubility combinations such as acrylamide/benzene (0.3% (w/w)) were found effective, presumably because of high chemical potential of solutes in the solution. The wide variety of guest species thus attained is expected to be useful in applications such as drug delivery or composite material preparation.

Introduction

β-Chitin, the rarer crystalline allomorph of chitin, occurs in squid pen, *Aprodite* chaetae, lorica of sessile ciliate, pogonophore tubes, and diatom spines.[1−6] This allomorph has been found to form crystalline complexes incorporated with small polar molecules such as water,[7−9] aliphatic alcohols,[10] and aliphatic amines.[11] These complexes are characterized by widening of the spacing along the *b*-axis of β-chitin due to intercalation of the guest species between chitin's molecular sheets formed by the stacking of planar acetylglucosamine moieties. This phenomenon apparently results from the high anisotropy of β-chitin's structure, i.e., the lack of hydrogen bonding between the sheets.[12] A notable feature of this complexation is that aliphatic alcohols[10] and aliphatic mono- and diamines[11] cause widening of chitin sheet spacing proportional to the length of alkyl chains. As chitin can be metabolized in human body, this phenomenon provides a potential usefulness in biomedical materials processing.

For such developments, however, more knowledge about the phenomenon is needed, especially for diversifying the type of guest species. The procedure of complexation reported so far involves immersion of β-chitin in a pure liquid of the guest. This method can be applied only to those guests having relatively low melting points, but many compounds of practical interests have high melting points and cannot be handled as liquid under moderate conditions. Therefore, we here developed a complexation technique based on solution treatments. Since this technique involves high selectivity in interaction with chitin for solute and solvent, basic aspects of guest selectivity by β-chitin were explored as well.

Experimental Section

β-Chitin. A high-concentration culture of diatom *Thalassiosira weissflogii* was supplied by Yamaha Motor Co. The culture suspension was lightly homogenized by a kitchen mixer for several seconds to effect dislodging of chitin spines from diatom cells. The homogenate was centrifuged at about 2000*g* for sedimenting the cells while chitin spines remained in supernatant. Then the supernatant was centrifuged at about 19 000*g* for collecting chitin as pellet. Recovered chitin was treated successively with 5% KOH (25 °C, overnight), methanol (65 °C, 2 h), 0.34% $NaClO_2$ (pH 4.9, 70 °C, 6 h), 0.1 N HCl (boiling, 1 h), and finally 1% HF (25 °C, overnight), with centrifugal rinsing with water after each. Purified chitin was freeze-dried and kept in a desiccator.

Oriented β-Chitin Fiber. For obtaining fiber X-ray diffraction data, β-chitin fiber was prepared as follows [adapted from Blackwell[7]]: Approximately 5 mg of dry chitin was dispersed in 7 mL of water and mixed with 3 mL of 1% fibrinogen solution [Wako Pure Chemical] in 3% sodium chloride. This mixture was supplemented by several drops of concentrated aqueous thrombin solution, immediately spread in a glass Petri dish to form approximately 3 mm thick layer, and allowed to stand at room temperature. When the layer formed a soft gel (after several hours, depending on temperature and amount of thrombin), it was cut into a 50 mm × 5−10 mm strip and slowly stretched by hands to about 3 times with continuous removal of water by contacting a filter paper. This stringlike specimen was softened by immersing in 1% KOH and further stretched to 1.5−2 times. The string was cut into 20 mm long pieces, and a proper number of pieces were bundled and inserted into a glass capillary of 1 mm inner diameter. Fibrinogen in the specimen was removed by alternately injecting 5% KOH solution and water into the capillary several times. The specimen was finally dried in a vacuum at 120 °C.

Preparation of Complexes. *1. Phenylenediamine Complex.* Pure liquid treatment: *o*-, *m*-, or *p*-phenylenediamine was

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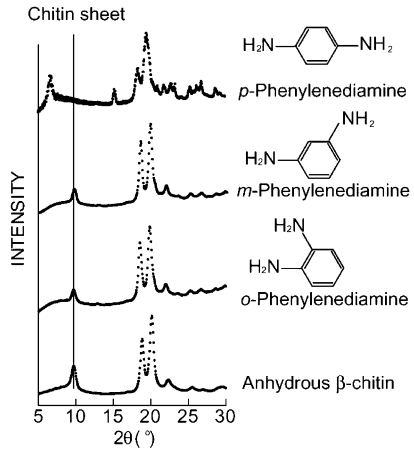

Figure 1. Equatorial X-ray diffraction profiles of β-chitin samples treated with pure liquids of *o*-, *m*-, and *p*-phenylenediamine. Only the *para* isomer gave the complex as seen by inward shift of chitin sheet. Small peaks at ca. 15° and above 20° are caused by excess guest.

heated to above the melting point (70−150 °C), and the anhydrous β-chitin specimen was soaked therein. After keeping for 30 min the specimen was removed, and extra liquid was removed quickly by pressing the specimen between filter papers. For comparison, *m*-xylenediamine, which has methylene groups between benzene ring and amino groups, was examined as complexing agent similarly as above at room temperature.

Solution treatment: *p*-phenylenediamine was dissolved in water, ethanol, acetone, dioxane, or benzene, all to make 1% (w/w) solutions. The anhydrous or hydrate form of β-chitin specimen was soaked in the solution for 30 min at 25 °C, except for benzene solution, which was handled at 50 °C.

β-Chitin hydrate was soaked in various concentration of *p*-phenylenediamine/dioxane solution (1−15% (w/w)) for 30 min at 25 °C.

2. Aliphatic Amine Complexes. Equimolar mixtures of (a) primary, secondary, and tertiary hexylamines, (b) hexylamine and octylamine, and (c) hexylamine and pentamethylenediamine were prepared. Anhydrous β-chitin specimen was soaked in the mixture at 25 °C for 30 min.

3. Complexation with High Melting Point Compounds by Solution Treatment. (a) β-Chitin-ethanol complex was soaked in 0.3% (w/w) acrylamide/benzene solution at room temperature. (b) β-Chitin hydrate was soaked in 0.2% (w/w) *p*-aminobenzoic acid/benzene solution at 50 °C. (c) β-Chitin hydrate was soaked in 7% (w/w) glucose/pyridine solution at room temperature. In all cases the specimen was made free of the excess solution as much as possible by compression between filter papers followed by vacuum-drying at 25 °C.

Characterization of Complex. *Fiber X-ray Diffraction.* X-ray diffraction diagrams were obtained by using nickel-filtered Cu Kα radiation (λ = 0.154 18 nm) from a rotating anode X-ray generator, RotaFlex RU-200BH (Rigaku), operated at 50 kV and 100 mA. The incident beam was perpendicular to the fiber axis of the specimen mounted in a vacuum camera. The diffraction pattern was recorded on an imaging plate (FUJIX BAS300UR, Fuji Film) and was read with RAXIS DS3 (Rigaku).

Gas Chromatography. For determining the host−guest ratio of the complex, some complex specimens of known weight was immersed in methanol, and extracted guest was quantified by gas chromatography. Measurements were conducted by a Shimadzu GC-14B with a Varian CP-Sil 8CB column. To verify that all guest molecules were extracted, the sample was examined by X-ray diffraction.

Results and Discussion

Incorporation of Phenylenediamine Isomers. Figure 1 shows the equatorial X-ray diffraction profiles of β-chitin treated with *o*-, *m*-, and *p*-phenylenediamines. The innermost peak represents the reflection by stacked chitin sheets,[10,11] whose spacing increases by intercalation with guest molecules. Of the three isomers, only *p*-phenylenediamine showed a shift of the peak, i.e., for 2θ = 9.63°−6.82°, corresponding to the change in lattice spacing of 0.919−1.296 nm. The latter value lies between those of trimethylenediamine and tetramethylenediamine complexes,[11] implying an arrangement of *p*-phenylenediamine between chitin sheets similarly to the case of type I alkyl diamine complexes. On the other hand, *m*-xylenediamine could be incorporated despite the *meta* geometry. These results indicate that the isomer selectivity for phenylenediamine is due to the rigid arrangements of amino groups, which make the *m*- and *o*- isomers difficult to be packed between chitin sheets by forming hydrogen bonds with chitin molecules.

Selectivity for Aliphatic Amines. When β-chitin was treated with pure liquid of primary, secondary, or tertiary amine with the same main chain (*n*-hexylamine, *N*-methyl-*n*-hexylamine, *N*,*N*-dimethyl-*n*-hexylamine), a complex formed only with primary and secondary amines and not with tertiary amine. Treatment with an equimolar mixture of these three species gave a complex having sheet spacing between those with primary and secondary amines (Figure 2A). Gas chromatography of the guest extracted from this complex gave the ratio of primary:secondary:tertiary amines as 68:32:0. This behavior seems to result from the steric hindrance by substituent groups on amino groups against hydrogen bond formation with chitin.

Treatment of β-chitin with equimolar mixture of octylamine and hexylamine gave a complex with sheet

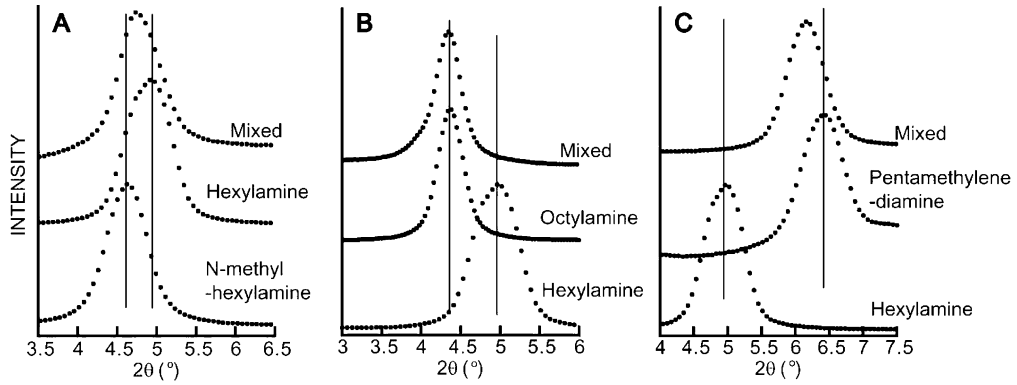

Figure 2. Equatorial X-ray diffraction profiles of β-chitin−amine complex swollen in mixed solution: (A) primary, secondary, tertiary hexylamine; (B) pentylamine and octylamine; (C) heptylamine and hepthamethylenediamine.

Table 1. Uptake of *p*-Phenylenediamine by *β*-Chitin from 1% (w/w) Solutions

solvent	from anhydrous *β*-chitin[a]	from *β*-chitin hydrate[a]	guest solubility at 25 °C (wt %)[b]
water	−	−	1.0
ethanol	+	++	5.7
acetone	−	+	12.4
dioxane	−	+	24.0
benzene[c] (50 °C)	−	++	1.2 (50 °C)

[a] ++, complete complexation; +, partial complexation; −, no complexation. [b] Determined gravimetrically. [c] Since solubility in benzene is too low at room temperature, treatment was done at 50 °C.

spacing same as that of octylamine complex (Figure 2B). Gas chromatography of the extract gave an octylamine−hexylamine ratio of 85:15. In this case, therefore, octylamine determines the sheet spacing, and hexylamine molecules are somehow embedded in the space formed by octylamine intercalation.

As Figure 2C shows, the treatment with an equimolar mixture of hexylamine and pentamethylenediamine gave a complex with sheet spacing of 1.434 nm, which is close to that for the pentamethylenediamine, 1.382 nm (vs 1.794 nm for hexylamine). Gas chromatography of the extract gave the diamine−monoamine ratio of 93:7. This selectivity is apparently due to stronger interaction of diamine with the chitin molecules.

Guest Uptake from Solution. The guest selectivity described above can be utilized for expanding the type of guest species in the complexation; i.e., inert species can be used as solvents for active species that have high melting point and cannot be applied to *β*-chitin as pure liquid. To demonstrate effectiveness of this strategy, we examined complexation with approximately 1% (w/w) *p*-phenylenediamine solutions in several solvents. At the same time, the influence of the starting form of *β*-chitin, i.e., anhydrous or hydrate, was examined. As Table 1 shows, the hydrate was a better starting material than anhydrous chitin. This behavior is understandable as the effect of preliminary loosening of the chitin crystal. As a result, the *p*-phenylenediamine complex was formed from solutions with ethanol, acetone, dioxane, and benzene as detected by X-ray diffraction.

It is notable that relatively low-concentration solutions of *p*-phenylenediamine in ethanol and benzene gave complete complexation (Figure 3). This behavior implies that the governing factor for the guest uptake is the chemical potential of solute, which must be positively dependent on the concentration relative to saturation. This interpretation is supported by the change in the degree of complexation by *p*-phenylenediamine/dioxane solutions of various concentrations (Figure 4). Since the saturation concentration is 24% (w/w), complete complexation of *β*-chitin required more than 15%. Therefore, an effective solvent for our purpose may not necessarily be a good solvent for the guest. This situation is advantageous, since it can reduce required amount of the guest, which may be valuable chemicals in applications such as drug delivery. The only exception to this tendency was water. This is presumably a result of water's high affinity to chitin to form hydrates.

The solution treatment broadens significantly the type of guest, since many compounds having high melting points can be applied in this way. To demonstrate its usefulness, Figure 5 shows successful complex

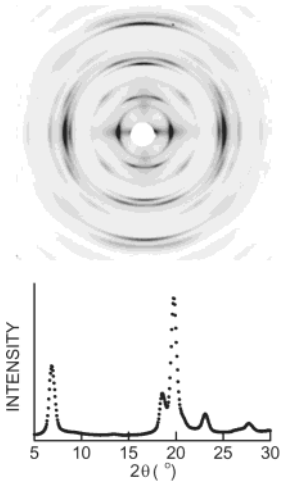

Figure 3. X-ray diffraction pattern and equatorial profile of *β*-chitin−*p*-phenylenediamine complex prepared by treating *β*-chitin hydrate with 1% (w/w) *p*-phenylenediamine/ethanol solution.

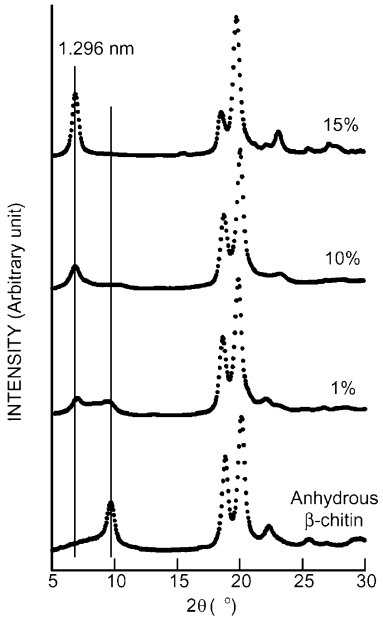

Figure 4. Equatorial X-ray diffraction profiles of *β*-chitin complexes prepared in various concentration of *p*-phenylenediamine/dioxane solution.

formation of *β*-chitin with acrylamide (mp 86 °C) in benzene (0.3% (w/w)), giving a lattice spacing of 1.333 nm, *p*-aminobenzoic acid (mp 186 °C) in benzene (0.2% (w/w)), giving a lattice spacing of 1.307 nm, and D-glucose (mp 146 °C) in pyridine (7% (w/w)), giving a lattice spacing of 1.272 nm. The patterns in Figure 5 contain reflections from *β*-chitin anhydrous and/or hydrate form, indicating incompleteness of the complexation. Their intensities, however, are weak enough compared to those of complexes. Our experience suggests that complete complexation would be attained by improving the treatment conditions. Formation of glucose complex was confirmed by two additional experiments: (i) Though pyridine itself forms a complex with *β*-chitin through pure liquid treatment, the lattice spacing of the complex was 1.470 nm (diffraction data not shown), clearly different from 1.272 nm. (ii) The glucose complex could also be formed by immersing the *β*-chitin−dimethyl sulfoxide complex (spacing 1.416 nm)

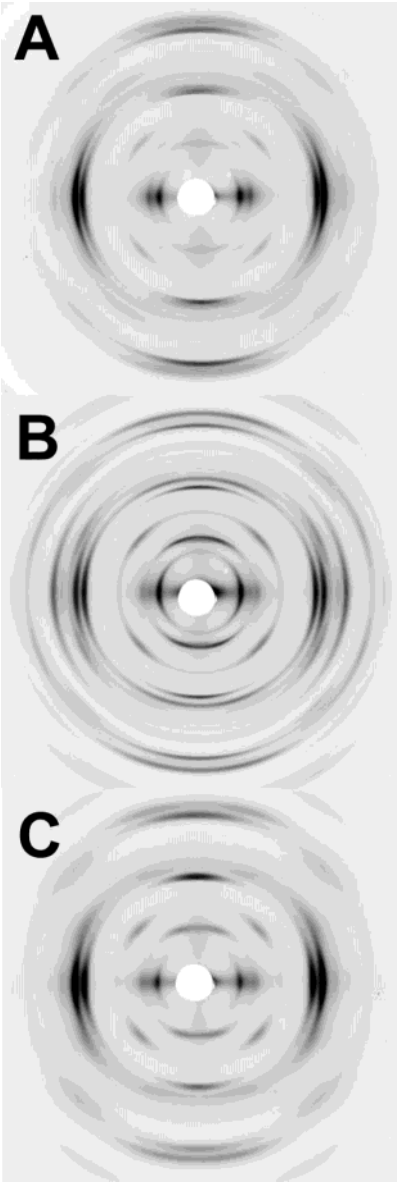

Figure 5. X-ray diffraction patterns of β-chitin complexes prepared by solution treatment: (A) acrylamide; (B) *p*-aminobenzoic acid; (C) D-glucose.

in molten glucose at 160 °C, giving almost the same sheet spacing as above, 1.277 nm.

All complexes described in this paper revert to initial β-chitin by washing with water and drying. This reversible complexation of β-chitin with these crystalline compounds attracts attention relating to possible applications; i.e., complexation with acrylamide provides the possibility of template polymerization, and that with *p*-aminobenzoic acid or glucose, both being biologically active molecules, could lead to utilization in drug delivery systems.

Conclusions

Intercalating uptake of small molecules by β-chitin has characteristic selectivity for guest species, and this feature sets the basis for the technique of using solutions for complexation treatment. The solution treatment was found to be effective even with low-concentration solutions in poor solvents. Observed features significantly widen the scope of guest species for β-chitin complexation and provide potential usefulness of the phenomenon for practical applications such as template polymerization, composite material processing, or biomedical materials.

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