Esterification of β-Chitin via Intercalation by Carboxylic Anhydrides

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β-chitin is known to form intercalation complexes with aliphatic alcohols and amines. We found that it also forms complexes with carboxylic anhydrides. When the β-chitin−acetic anhydride complex was heated to 105 °C, the hydroxyl groups of chitin were acetylated by a host−guest reaction, maintaining the host's crystal structure. Structures of complex and acetylated products were analyzed by X-ray diffraction, ^{13}C CP/MAS NMR, and infrared spectroscopy. The maximum degree of substitution (DS) was close to 1.0, suggesting regioselective esterification at the C6 position of chitin. Partially acetylated β-chitin with a DS of 0.4 could incorporate various guest species that are difficult to be incorporated by original β-chitin. In contrast, β-chitin acetate with a DS of 1 lost the ability to form a complex. Intercalation complexes of β-chitin with cyclic anhydrides (succinic and maleic) also underwent esterification by heating, and the products with a DS of ∼1 dissolved in aqueous alkali, apparently as the result of the dissociation of introduced carboxyl groups. These phenomena are potentially useful in controlling the complexation ability of β-chitin and the preparation of regioselectively esterified chitin derivatives.

Introduction

Many crystalline solids are known to form intercalation complexes by incorporating small molecules within the crystal lattices. Well-known examples of hosts are inorganic crystals such as graphite, clay, and metal oxides. In natural polysaccharides, cellulose I,[1−3] α-chitin,[4] and β-chitin are known to form such crystalline complexes. While cellulose I and α-chitin can incorporate only strong intercalators such as small diamines, β-chitin is known to be active for a wider variety of guest species, including water,[5,6] higher alcohols,[7] monoamines/diamines,[8] and even glucose.[9] β-chitin is the rarer crystal allomorph of chitin, occurring in a limited number of species such as squid pen, pogonophore tubes, and diatom spines.[10] In contrast to the antiparallel arrangement of α-chitin, β-chitin's structure is characterized by the parallel-chain arrangement, which lacks hydrogen bonding between the stacked molecular sheets.[11] This structure is considered to be the cause of the intercalation capability mentioned above.[12] To further understand this phenomenon and explore the possibility of its utilization, here we studied the intercalation of β-chitin by carboxylic anhydrides and their host−guest reactions.

Experimental Section

Chemicals. All the reagents were of chemical grade, from Wako Pure Chemicals, unless stated otherwise. Glutaric anhydride was obtained from Tokyo Kasei Co. Resin-deionized water was used throughout the experiment.

Chitin Sample. A high-concentration culture of *Thalassiosira weissflogii* was provided by Yamaha Motor Co. The purification of β-chitin from the culture and the preparation of oriented chitin specimen for X-ray diffraction were carried out as described previously.[8] Briefly, β-chitin spines separated from diatom bodies by vigorous shaking were collected from the upper part of the suspension after weak centrifugation. Chitin spines were successively treated with methanol (80 °C, 2 h), 5% KOH (25 °C, overnight), 0.34% $NaClO_2$ (buffered to pH 4.0, 70 °C, 6 h), 0.1 N HCl (boiling, 1 h), and finally 1% hydrogen fluoride (25 °C, overnight), with rinsing with water and recovery by centrifugation after each step. The purified chitin sample was freeze-dried to give a fluffy solid. Although we did not determine the molecular weight and degree of deacetylation of this chitin sample, it is likely to have had a fairly high molecular weight and a low degree of deacetylation, based on the high perfection of the crystal seen by X-ray diffraction (Figure 1A).

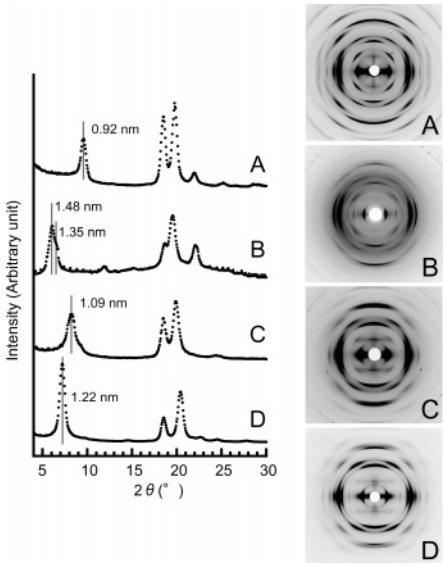

Figure 1. X-ray diffraction patterns and equatorial profiles. (A) anhydrous β-chitin. (B) β-Chitin−acetic anhydride complex. (C) Acetylated β-chitin after 10 min of heating at 105 °C. (D) Acetylated β-chitin after 2 h of heating at 105 °C.

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Table 1. Complexation of β-chitin with Carboxylic Anhydrides and Esterification Therefrom

carboxylic anhydride	complexation condition	thermal esterification conditions[a]	010 *d*-spacing of complex (nm)	molar ratio of guest[c]	010 *d*-spacings of ester (nm)	solubility of ester
acetic	immersion of DMSO complex in liquid anhydride	105 °C for 2 h in liquid anhydride	1.48 (type II)	1.68	1.22	dichloroacetic acid
			1.35 (type I)[b]	1.29		
propionic	complex not formed					
succinic	immersion of octanol complex in liquid anhydride	150 °C for 24 h in liquid anhydride	1.42	1.60	1.39	alkali
maleic	immersion of octanol complex in liquid anhydride	150 °C for 24 h in liquid anhydride	1.40 (type II)	1.91	1.34	alkali
			1.28 (type I)[b]	1.49		
glutalic	immersion of octanol complex in liquid anhydride	150 °C for 24 h in liquid anhydride	1.51 (type II)	1.73	1.30	[d]
			1.38 (type I)[b]	1.16		
pyromellitic dianhydride	immersion of octanol complex in sat. solution of pyromellitic anhydride/acetone	not examined	1.31	0.83	[d]	[d]

[a] Via complexation. For acetic, succinic, and maleic anhydrides, the DS of the ester reached approximately 1. [b] Type I complex is obtained by air-drying the Type II complex overnight at room temperature. [c] Estimated from the increase in lattice volume. The real number seems to be 2.0 for Type II, and 1.0 for Type I. [d] Not examined.

For obtaining oriented specimen, 5 mg of dry chitin was dispersed in 7 mL of water and mixed with 3 mL of 1% fibrinogen solution in 3% aq sodium chloride. The mixture was coagulated by adding several drops of concentrated aq thrombin solution to form a soft gel. The gel was stretched by hand, then treated with 5% KOH for removing fibrinogen. The specimen was finally washed with water and dried to give an oriented β-chitin fiber specimen. For Fourier transform infrared (FTIR) measurements, a thin film was prepared by dispersing the chitin sample in water by sonication to give a 0.7 mg/mL suspension, which was cast on a Teflon plate.

Complex Formation and Esterification with Acetic Anhydride. The experiments were conducted at room temperature unless otherwise stated. Approximately 1 mg of oriented fiber or film specimen of β-chitin was immersed in water and kept for approximately 2 h (formation of β-chitin hydrate). The material was made free of excess water by being pressed between filter papers. The specimen was successively immersed in 2 mL of dimethyl sulfoxide (DMSO) for 2 h (formation of the β-chitin−DMSO complex) and 2 mL of acetic anhydride for 2 h (formation of the β-chitin−acetic anhydride complex), with removal of excess liquid with filter papers after each step. The β-chitin−acetic anhydride complex was heated in excess acetic anhydride for 0−96 h at 40−105 °C (acetylation of chitin), then washed with water and dried in vacuo.

Complex Formation and Esterification with Cyclic Carboxylic Anhydrides. The β-chitin sample was immersed in hexylamine and kept for approximately 2 h (formation of the β-chitin−hexylamine complex), then immersed in 2 mL of 1-octanol for 2 h (formation of the β-chitin−octanol complex). The β-chitin−octanol complex was immersed in an excess of succinic anhydride at 125−150 °C for 0−48 h (succinylation of chitin), then washed with acetone and dried in vacuo. For other carboxylic anhydrides, see Table 1 for details.

Preparation of β-Chitin Diacetate. β-Chitin diacetate was prepared for the estimation of the degree of substitution (DS) by FTIR spectrometry. Heterogeneous acetylation of the β-chitin sample was carried out according to VanderHart et al.[13] The dry β-chitin sample was soaked in 90% acetic acid for 2 h and then in two exchanges of 100% acetic acid for l h each. The acetic acid-wet β-chitin was immersed in a 1:1 v/v mixture of acetic anhydride and toluene with a trace of perchloric acid as the catalyst. The reaction was allowed to proceed for 48 h at room temperature. The reaction was stopped by placing the specimen in 95% ethanol for several hours. After washing with ethanol, the sample was dried in vacuo at 120 °C overnight. The acetylated chitin was analyzed by FTIR. By this treatment, the OH stretching band (3200−3600 cm^{-1}) totally disappeared, showing that O-acetylation was complete (DS 2.0).

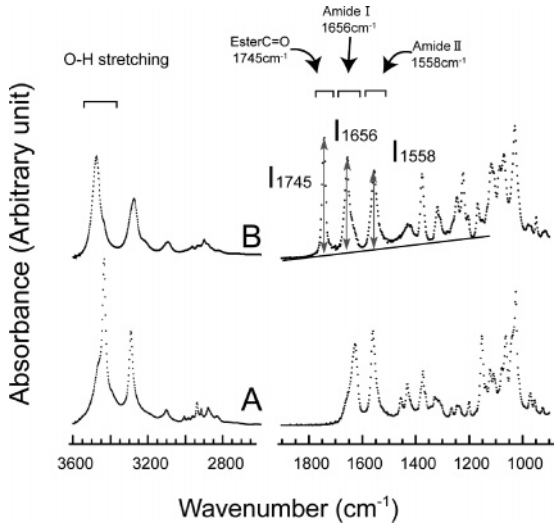

Figure 2. FTIR spectra of (A) anhydrous β-chitin, and (B) β-chitin monoacetate obtained by heating the complex at 105 °C for 2 h.

X-ray Fiber Diffraction. A glass capillary-sealed wet specimen (guest-saturated complex) or bare bundle specimen (vacuum-dried complex) was subjected to X-ray diffraction by a transmitting beam from a rotating anode X-ray generator, RotaFlex RU-200BH (Rigaku), operated at 100 mA and 50 kV, using nickel-filtered Cu Kα radiation ($\lambda = 0.15418$ nm). The diffraction pattern was recorded on an imaging plate (FUJIX BAS300UR, Fuji Film) and read by an RAXIS DS3 (Rigaku).

FTIR Measurements. Infrared spectra were measured by a Nicolet Magna 860 FTIR spectrometer. Film samples were analyzed under a flux of dry air. All spectra were recorded in transmission mode with an accumulation of 32 scans and a resolution of 4 cm^{-1}.

Estimation of DS from FTIR Spectra. The DS was estimated based on the method of Ando et al.[14] with some modification. The peak height was determined for amide I (1656 cm^{-1}), amide II (1558 cm^{-1}), and ester C═O stretching (1745 cm^{-1}) by using a straight baseline drawn from 1850 to 1190 cm^{-1} (see Figure 2B). Two absorbance ratios, D_I or D_{II}, were defined by

$$D_I = I_{1745}/I_{1656} \quad (1)$$

$$D_{II} = I_{1745}/I_{1558} \quad (2)$$

DS values, R_I and R_{II}, were calculated as follows:

$$R_I = 2D_I/D_{I-diacetate} \quad (3)$$

where $D_{I-diacetate}$ and $D_{II-diacetate}$ are the corresponding ratios for chitin

$$R_{II} = 2D_{II}/D_{II-diacetate} \quad (4)$$

diacetate.

^{13}C CP/MAS NMR. The samples were introduced into airtight sealed 4-mm BL-type ZrO_2 rotors. ^{13}C NMR spectra (100 MHz) in the solid state were recorded with a Bruker Avance spectrometer equipped with a 4-mm BL-type probe. The spectra were acquired at room temperature under a 80 kHz proton dipolar decoupling field, matched cross-polarization (CP) fields of 80 kHz, a proton 90° pulse of 2.5 μs, and magic angle spinning (MAS) at a spinning speed of 6 kHz. The CP transfer was achieved using a ramped amplitude sequence (RAMP−CP) for an optimized total contact time of 2 ms. The sweep width was of 50 000 Hz to avoid baseline distortion with 2994 TD points, and the Fourier transformation was achieved without apodization over 8k points. The repetition time was 4 s, and an average of 10 000 scans was acquired for each spectrum. The ^{13}C chemical shifts were determined relative to the carbon chemical shift of the glycine carboxyl group (176.03 ppm).

Transmission Electron Microscopy. A drop of dilute suspension of β-chitin or acetylated β-chitin was mounted on a carbon-coated grid (Okenshoji Co.). The specimen was examined by phase contrast with a JEOL 2000 EX operated at 200 kV.

Results

Intercalation of Acetic Anhydride into β-Chitin. The intercalation of β-chitin by acetic anhydride was not possible by direct immersion of anhydrous β-chitin in the latter liquid, but was possible by the guest-exchange method using DMSO as the intermediate guest. The occurrence of intercalation could be detected by the shift in the 010 equatorial reflection of the X-ray diffractogram of β-chitin. Complexation was also possible by using hexylamine or octanol as the intermediate guest. At room temperature, the complex did not undergo further changes when kept under wet conditions. Contact with solvents such as water or ethanol readily caused extraction of guests. Also, long standing under open conditions or short heating over 50 °C caused the release of the guest species.

Although the unit cell parameters of the chitin−acetic anhydride complex have not been determined, here we assume them to be one-chain monoclinic for convenience, and refer to the innermost equatorial reflection as 010. The shift of this reflection obviously indicates an expansion of the chitin sheet spacing by intercalation. In this course, the 010 spacing changed successively from that of the anhydrous form (0.92 nm, Figure 1A), the hydrate (1.11 nm), to the DMSO complex (1.40 nm), and the acetic anhydride complex (1.48 nm, Figure 1B). As in the case of water[5,6] or some diamines,[8] there were two types of acetic anhydride complexes, depending on the treating conditions; that is, the complex with a 010 spacing of 1.48 nm changed to the complex with a 1.35 nm spacing by standing in air, say, overnight at room temperature. Assuming that the density of the guest in the packed state is the same as that of the bulk liquid, one can estimate the stoichiometric ratio of the complex from the increase in the lattice volume where the guest molecules are packed. In the previous study, this method gave reasonable values for several guest species.[8] In the present case, this calculation gave an acetic anhydride−chitobiose ratio of 1.68 for the complex with 1.48 nm spacing, and 1.29 for the complex with 1.35 nm spacing. Since these values are not close to integers, they may not follow stoichiometry as did the previous examples. Still, the formation of the two types of complexes was reproducible. This behavior may indicate the occurrence of nonintegral inclusion ratios.

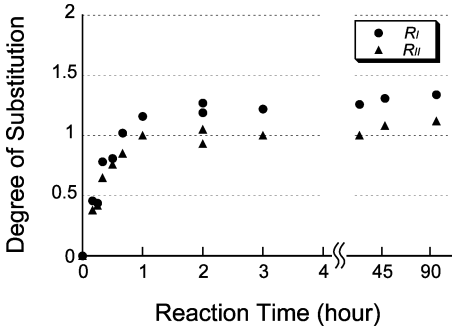

Figure 3. DS of β-chitin acetate in the course of heating the β-chitin−acetic anhydride complex at 105 °C. ● and ▲ show the DS calculated from the ester C═O stretching band.

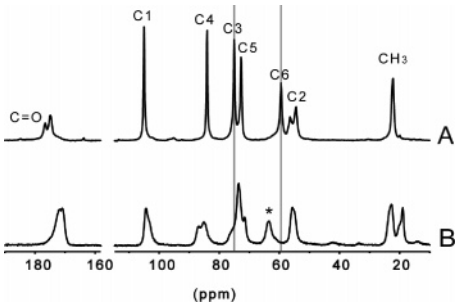

Figure 4. Solid-state ^{13}C NMR spectra of (A) anhydrous β-chitin and (B) β-chitin monoacetate obtained by heating the complex at 105 °C for 2 h. Shifted C6 is marked with (*) in panel B.

Thermal Acetylation of β-Chitin through Acetic Anhydride Complexation. When the β-chitin−acetic anhydride complex was heated over 40 °C with excess guest liquid, the hydroxyl groups of chitin were acetylated, as evidenced by the change in the FTIR spectrum (Figure 2). The O−H stretching bands of the original chitin at 3475 and 3435 cm^{-1} decreased significantly in the heat-treated complex. On the other hand, the ester C═O stretching band at 1745 cm^{-1} could be observed separately from the C═O bands of chitin's amide I (1656 cm^{-1}) and amide II (1558 cm^{-1}). The intensity of the 1745 cm^{-1} band was determined and used for calculation of the DS. Figure 3 shows the increase in DS against the heating time at 105 °C. The DS seems to level off at 1.0 after 1 h.

Chitin has two hydroxyls in each repeating unit at C3 and C6. With a DS of 1.0 by the present treatment, it is highly likely that esterification has occurred at one of these positions selectively. To determine the site of reaction, we analyzed the specimen by ^{13}C CP/MAS NMR (Figure 4). The assignment of signals for β-chitin followed Kono.[15] In a comparison of the spectra of the esterified sample and those of the original β-chitin, the C6 resonance showed a large shift to lower field, from 59.4 to 63.3 ppm. This strongly suggests that the primary hydroxyl at C6 had been esterified. The other changes, that is, the slight shifts and change in peak shapes of C3, C4, and amide C═O carbon, are considered as resulting from the conformational change due to esterification.

The X-ray diffractograms of esterified samples (Figures 1C and 1D, heating time 10 min and 2 h, respectively) showed changes in the 010 spacing, while other reflections (100, 1-10, and 002) were nearly unchanged. These features indicate that the sheet structure of β-chitin was maintained through acetyl-

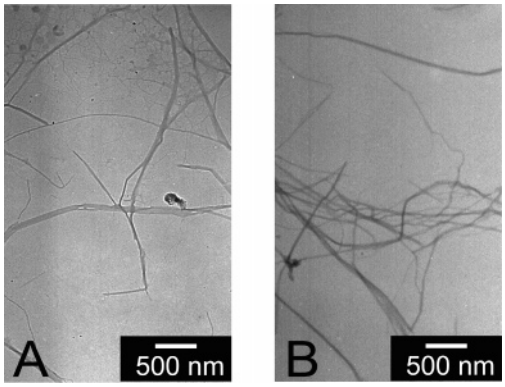

Figure 5. TEM micrographs of (A) anhydrous β-chitin and (B) β-chitin monoacetate obtained by heating the complex at 105 °C for 2 h.

ation. This was also evidenced by the retention of the original microfibrillar morphology in electron microscopy (Figure 5).

Properties of β-Chitin Acetate. *Solubility.* The acetylated β-chitin, presumably monoacetate, was tested for solubility in various solvents, including common organic liquids and chitin solvents such as LiCl/DMAc and $CaCl_2 \cdot 2H_2O$/MeOH.[16] Similarly to α-chitin acetate (DS 0.3−2.0),[17] the product dissolved only in dichloroacetic acid or formic acid, with a certain degree of de-esterification (data not shown). The low solubility of chitin monoacetate may result from the presence of strong intermolecular interactions, possibly due to dense packing of protruding side groups, that is, acetate and the original acetamide groups.

Intercalation Behavior. The regioselective acetylation maintaining the β-chitin's sheet structure is expected to alter its intercalation behavior. The acetate of DS 0.4 could be directly intercalated by propionic acid, 1-butanol, and acetic anhydride, which do not intercalate β-chitin directly. In contrast, the acetate of DS 1 lost its complexation ability, even with strong intercalators such as aliphatic diamines. This feature is consistent with the low solubility described above.

Esterification via Intercalation of Other Carboxylic Anhydrides. Complexation of β-chitin with carboxylic anhydrides other than acetic anhydride and thermal esterification therefrom were examined. The results are summarized in Table 1. While propionic anhydride did not form a complex with β-chitin, several cyclic anhydrides did. Succinic, maleic, and glutaric anhydride formed complexes and underwent thermal esterification, as indicated by changes in the 010 spacing. The β-chitin succinate and maleate prepared by this method gave a maximum DS of approximately 1.0, as in the case of acetic anhydride. Again, the esterification proceeded, retaining the β-chitin's sheetlike structure and microfibrillar morphology (data not shown). In contrast to the acetate, the esterified products from these cyclic anhydrides dissolved in aqueous alkali, apparently because of the introduction of free carboxyls attached to the ester moieties.

Discussion

Heating anhydrous β-chitin immersed in acetic anhydride (i.e., without complexation) resulted in slow and incomplete acetylation; it took more than 24 h of heating at 105 °C to give a DS of 0.4. An additional feature of the reaction without complexation was the occurrence of two separate reflections in the X-ray diffraction, corresponding to 0.92 nm (unreacted region) and 1.22 nm (reacted region). This indicates that the acetylation proceeds slowly and inhomogeneously, probably from the surface of the crystal. In contrast, acetylation via β-chitin complexation proceeded very readily. The DS reached 0.4 in 10 min, and then 1.0 in approximately 60 min, at 105 °C. In the course of reaction, the 010 reflection gave a single peak, which shifted gradually to a greater angle (Figure 1-C). This means that the reaction proceeded uniformly throughout the crystal. This must result from close molecular contacts between chitin with acetic anhydride.

Several studies have been conducted on chitin esterification.[14,17,18] Most of these were for α-chitin, which required homogeneous or severe conditions. Kurita et al. reported that β-chitin was advantageous over α-chitin in chemical modifications because of β-chitin's swellability.[18] Our present results, that is, the direct incorporation of reactants into the crystal and subsequent rapid reactions, are a manifestation of the high reactivity of β-chitin, providing an effective method of chitin derivatization. Also, the change in complexation ability by partial esterification may be useful in the application of β-chitin for biomedical fields; for example, it would be useful as a drug delivery agent or an imaging contrast medium, if the β-chitin microcrystals can incorporate pharmaceutically active chemicals or heavy metal ions/particles.

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