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ORIGINAL PAPER

Crystalline alignment of metal ions templated by β-chitin ester

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Abstract The highly crystalline β-chitin from diatom *Thalassiosira weissflogii* was esterified via intercalation with succinic anhydride followed by simple heating, maintaining the original crystalline order. Due to the introduced free carboxyl groups, the chitin ester crystal showed ion exchange ability for metal cations in aq. solution. Heavy metal cations such as Pb^{2+} bound to the β-chitin succinate gave characteristic X-ray diffraction patterns, indicating regular alignment of metal ions. Such materials represent a new type of organometallic architecture, possibly leading to novel functionalities.

Keywords Chitin · Intercalation · Esterification · Ion exchange

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Introduction

Regular alignment of metal ions can arise in organic or inorganic salt crystals. Such materials are usually of low molecular weight, and cannot be handled as solid due to solubility to water or organic solvents. Organic polymers, on the other hand, can be constructed as water-insoluble materials, but many of them are noncrystalline because of molecular flexibility and irregular structures. β-chitin, the rarer crystal form of chitin, linear polymer of β-1,4-linked *N*-acetylglucosamine, is known to be capable of incorporating various polar molecules such as water (Blackwell 1969; Sawada et al. 2012), aliphatic alcohols (Saito et al. 1998), and aliphatic/aromatic amines (Noishiki et al. 2003, 2004; Saito et al. 2007; Sawada et al. Sawada et al. 2013), forming characteristic intercalation crystalline compounds. One class of guest is carboxylic anhydrides, which subsequently undergo esterification specifically on hydroxymethyl groups of chitin by simple heating (Yoshifuji et al. 2006). Notably in our previous study, several cyclic anhydrides could be used as guest for esterification (Yoshifuji et al. 2006). Since the ring-opening

esterification with these cyclic anhydrides leads to the regular arrangement of free carboxyl groups in the crystal, the metal ion is expected to align regularly by cation exchange reaction with the β-chitin ester crystal as a template. Thus, we examined the ion exchange behavior of β-chitin ester with metal ions and analyzed structure of the products.

Experimental

Materials

β-chitin was obtained as spines of a marine centric diatom, *Thalassiosira weissflogii* (Dweltz et al. 1968). High-concentration cultures of the diatom were provided by YAMAHA Co. Ltd. (Japan). β-chitin was isolated from the culture as described elsewhere (Noishiki et al. 2003). Briefly, the cell culture suspension was shaken vigorously for detaching chitin spines from cells; the spines were collected as supernatant suspension after weak centrifugation (2,000 rpm, 5 min), and purified by successive treatments with methanol (65 °C, 2 h), 5 % aq. KOH (25 °C, overnight), 0.3 % aq. $NaClO_2$ (70 °C, 6 h), 0.1 N HCl (boiling, 1 h), and finally 1 % HF (25 °C, overnight). The material was thoroughly washed with water and freeze-dried.

The β-chitin sample was formed as oriented fiber for X-ray diffraction measurement by using fibrinogen gel-stretching technique (Blackwell 1969; Noishiki et al. 2004; Yoshifuji et al. 2006). Briefly, 5 mg of dry β-chitin was dispersed in 7 mL water and mixed with 3 mL of 1 % fibrinogen solution in 3 % *aq*. sodium chloride. Mixture was coagulated by adding several drops of concentrated *aq*. thrombin to form a soft gel slab. The chitin fibrils in the slab was uniaxially oriented by manually stretching the slab slowly to about 6 times of original length. The fibrinogen was removed by 5 % KOH treatment and water rinsing. The fiber specimen was inserted in a glass capillary for further treatments for esterification and ion exchange. The chitin sample was also formed as a randomly oriented film by filtration for infrared spectroscopy.

Esterification via intercalation

Purified chitin samples, either as oriented fibers or films, were soaked with water for about 5 min at room temperature to form β-chitin hydrate. After blotting-removal of excess water, the sample was soaked in 2 ml of dimethyl sulfoxide (WAKO) for 2 h at room temperature to form β-chitin-DMSO complex. The sample was then soaked by neat succinic anhydride (melting point at 115 °C) for 24 h at 140 °C for esterification. The product was washed with acetone and water, and dried in vacuo.

Ion exchange capacity of chitin succinate was determined by conductometric titration as follows: 21.6 mg of chitin succinate sample was soaked in 100 ml of deionized water and titrated by 0.1 M NaOH under conductivity monitoring.

Cation exchange of β-chitin succinate

Potassium acetate and Pb(II) acetate were used as salts, dissolved in distilled water to give 5 % (w/w) solutions. β-chitin succinate sample, in the form of oriented fiber or randomly deposited sheet, was immersed in these solutions, and then washed with water. For oriented fiber sample, this treatment was done by injecting the salt solution into the glass capillary holding the fiber. Successful incorporation of metal cations could be detected by increase of dry weight of the specimen.

X-ray diffraction

The oriented fiber specimen of esterified β-chitin or its ion-exchanged product was subjected to X-ray diffraction by transmitting beam of Ni-filtered Cu Kα radiation ($\lambda = 0.15418$ nm) from a rotating anode X-ray generator, RotaFlex RU-200BH (Rigaku, Tokyo, Japan) operated at 100 mA and 50 kV. Diffraction pattern was recorded on an imaging plate (FUJIX BAS300UR, Fuji Film, Tokyo, Japan) and read by RAXIS DS3 (Rigaku, Tokyo, Japan).

Fourier transform infrared (FT-IR) spectroscopy

Infrared spectra of esterified β-chitin were measured by a JASCO FTIR-615 Fourier Transform Infrared Spectrometer (JASCO, Tokyo, Japan). Spectra were recorded in ATR mode for thin films under dry nitrogen flow with accumulation of 128 scans and resolution of 4 cm^{-1}.

Transmission electron microscopy

Dilute suspension of chitin or its ester sample was dropped on a carbon-coated grid (Oken, Japan) and then dried in air. All transmission electron microscopy was

carried out with a JEM 2000EXII (JEOL, Tokyo, Japan) equipped with a CCD camera (Keen view, Olympus Soft Imaging Solutions, Münster, Germany) operated at 200 kV. An objective aperture of 20 μm was used for diffraction contrast imaging to eliminate all the diffracted beams from the crystalline areas. The diffraction contrast images were recorded on the CCD camera with 1 s exposure by using Minimum Dose System (MDS, JEOL, Tokyo, Japan) (Ogawa et al. 2011). Observed images were then analyzed by using iTEM software (Olympus Soft Imaging Solutions, Münster, Germany). The widths of 100 microfibrils of each specimen were measured for evaluation of width distribution.

Adsorption measurement of Pb^{2+} ion

Two milligrams of freeze-dried purified chitin and its succinate samples were dispersed in 10 ml of aqueous Pb(II) acetate (10–800 mg/ml) for 2 days at 25 °C. The concentration of Pb^{2+} ion in the supernatant liquid was determined using a metal assay kit based on chelate coloration (Metallo Assay, Metallogenics, Chiba, Japan).

Results and discussion

Esterification of β-chitin via intercalation

The β-chitin specimen, either oriented fiber or randomly deposited sheet, could undergo intercalation by succinic anhydride and subsequent thermal esterification maintaining their crystalline states. While the macroscopic appearance of the product did not change, X-ray diffraction analysis showed clear changes in crystal structure as in Fig. 1. The diffraction pattern after succinic anhydride treatment shows a shift of innermost equatorial reflection from $2\theta = 9.61°$ to $6.22°$ indicating the formation of intercalate. By heating the intercalate at 120 °C for 24 h, the corresponding peak shifted to 6.38°. These changes correspond to shift of sheet spacings of 0.92 nm (anhydrous β-chitin) to 1.42 nm (succinic anhydride intercalate), and to 1.39 nm (succinic ester) (Blackwell 1969; Nishiyama et al. 2011; Yoshifuji et al. 2006).

FT-IR spectra of anhydrous β-chitin and its succinate show corresponding changes as in Fig. 2. Heating of the intercalate at 140 °C for 24 h caused a significant decrease in the intensity of OH band at 3,400–3,500 cm^{-1} and appearance of C = O band at 1,714–1,735 cm^{-1}, suggesting the partial esterification of OH groups. Another indication of esterification was that the product was readily soluble in dilute aq. alkali such as 2 % NaOH. For acetylation via intercalation by acetic anhydride, the reaction was supposed to be specific to C6 position due to its greater accessibility than C3 (Yoshifuji et al. 2006). The same situation is likely for the present case of succinylation. Therefore this esterification reaction is considered to occur only at primary alcohol groups as shown in Scheme 1.

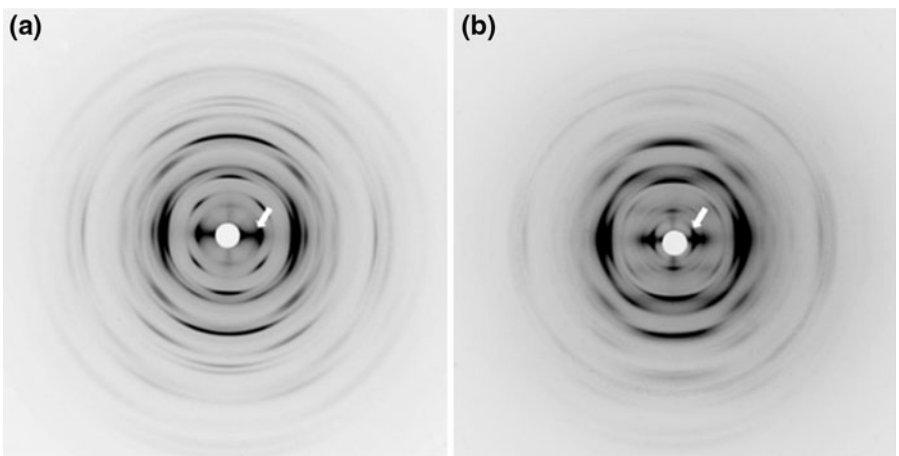


Fig. 1 X-ray fiber diffraction patterns of **a** anhydrous β-chitin and **b** β-chitin succinate in free COOH form. *Arrow* mark in **a** shows 001 reflection corresponding to 0.92 nm spacing between chitin molecular sheets. *Arrow* mark in **b** shows enlarged sheet spacing of 1.39 nm with intercalation by succinate

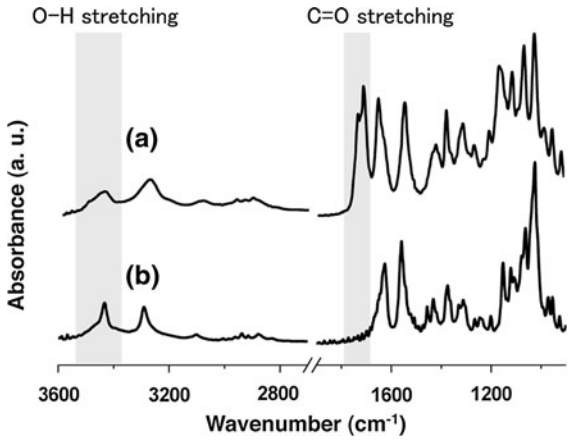


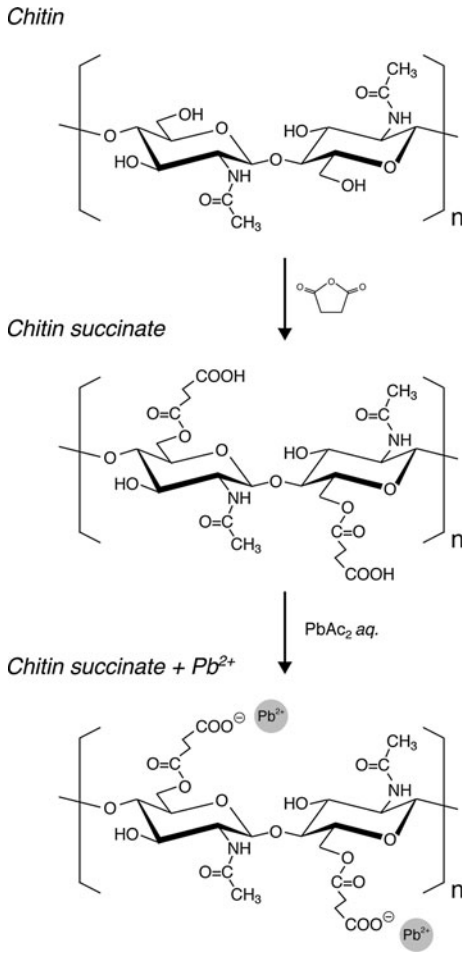
Fig. 2 FT-IR spectra of **a** anhydrous β-chitin and **b** β-chitin succinate. Decrease in OH band and appearance of C = O in **b** shows effective esterification by succinic anhydride

The degree of substitution of the β-chitin succinate was evaluated by conductometric titration (Fig. 3). Here the change in conductivity of aqueous suspension of β-chitin succinate clearly reflected the cation exchange of the bound succinate from free acid form to Na^+ salt. From this result the ion exchange capacity was determined to be 3.75 meq/g, corresponding to the degree of substitution of 0.99.

The ion exchange capacity of the succinate crystal was estimated by the adsorption measurement using Pb^{2+} ion. The equilibrium adsorption of Pb^{2+} ion was 0.12 mg/mg for original β-chitin, and 0.34 mg/mg for the succinate. Since the former is considered to be a non-specific adsorption onto the surface of microfibrils, the uptake of Pb^{2+} in the crystal of the succinate was determined as 0.22 mg/mg from difference between the two values, giving Pb^{2+}/chitobiose ratio of 0.65. Discrepancy of this value and 0.99 from the titration above may have resulted from difficulty of penetration of large Pb^{2+} ions into the crystal.

Structure of cation-exchanged β-chitin succinate

If the β-chitin succinate obtained here has selective substitution at C6 position, its ion exchange product is considered to have regular arrangement of metal cations in the chitin-derived crystal (Scheme 1). This was found actually the case as shown by X-ray diffraction. Fig. 4 shows the fiber diffraction pattern of Pb(II) salt of the β-chitin succinate obtained by treatment of the esterified sample with aq. Pb(II)



Scheme 1 Esterification of β-chitin with succinic anhydride, and ion exchange reaction with Pb^{2+} ions

acetate. The pattern showed basic similarity to that of β-chitin succinate (Fig. 1b), but very strong equatorial and meridional reflections appeared additionally. Since these reflections were too strong in the diagram by ordinary exposure, a low-exposure diagram was recorded as Fig. 4b, by 1/4 exposure time. Here the reflections from chitin crystal are nearly invisible, but three reflection pairs, two equatorial and one innermost meridional, are clearly visible. These reflections presumably result from periodical arrangement of Pb^{2+} ion, which has high electron density. The equatorial peaks correspond to 1.69 and 0.852 nm; the latter is likely to be the second order reflection of the former. The spacing of 1.69 nm is reasonable when compared to that of succinate in free form, 1.39 nm, since incorporation of large Pb^{2+} ion. The meridional 0 0 1 reflection corresponds to 1.032 nm,

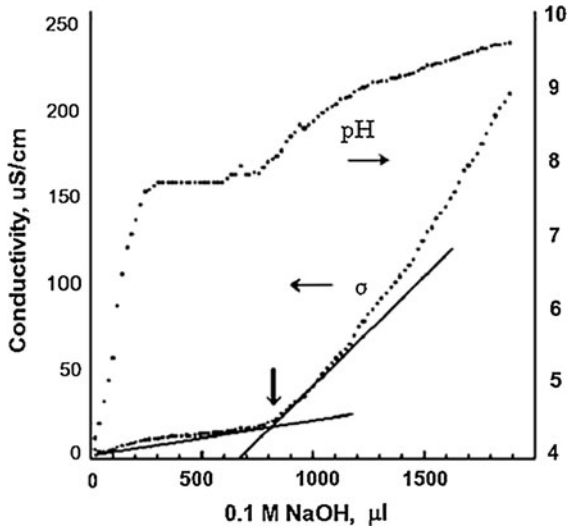


Fig. 3 Conductivity and pH titration curves of β-chitin succinate by 0.1 M NaOH from free acid form. Down *arrow* shows end point

close to fiber repeat distance of β-chitin, 1.038 nm; this discrepancy may have resulted from slight deformation of the chitin molecules in the course of esterification and binding of cation. Although a certain amount of Pb^{2+} ions is adsorbed nonspecifically on the surface of the microfibrils, this result indicates that in the crystalline region the Pb^{2+} ions bound to carboxyl groups of the β-chitin succinate form a regular array dictated by the host crystal. While the exact arrangement of the Pb^{2+} ions must be determined by rigorous crystal analysis, rough crystal structure and stoichiometry could be estimated based on the diffraction pattern: The strong 0 0 1 reflection indicates the absence of $P2_1$ symmetry along its fiber direction and inclusion of one Pb^{2+} ion per unit cell. This stoichiometric ratio between Pb^{2+} and chitobiose, 1.0 is consistent with the result from titration and the electroneutrality condition in the crystal.

Binding of Pb^{2+} ions to the β-chitin succinate was visualized by transmission electron microscopy. Figure 5 shows the electron diffraction contrast images of initial β-chitin, β-chitin succinate, and its Pb^{2+}-exchanged specimens. All these microfibrils were observed with strong dark contrast under this low dose condition, indicating their highly-crystalline nature. The average width of Pb^{2+}-exchanged specimen is the largest, followed in order by the succinate, and initial β-chitin. This tendency coincides with sheet spacing detected by X-ray diffraction.

Other heavy metal salts were also tested for ion exchange behavior. Since the succinic ester is a weak acid, only the metal cations with weak counter anions such as acetate are expected to cause cation exchange. In fact, treatment of the β-chitin succinate in 5 % (w/w) aq. $PbNO_2$ did not result in ion exchange as examined by X-ray diffraction. Other metal cations that were available as water-soluble acetate, such as K^+, Gd^{3+}, and UO^{2+}, underwent ion exchange as detected gravimetrically.

In summary, the β-chitin succinate prepared via intercalation of succinic anhydride shows ion exchange behavior maintaining the original crystalline

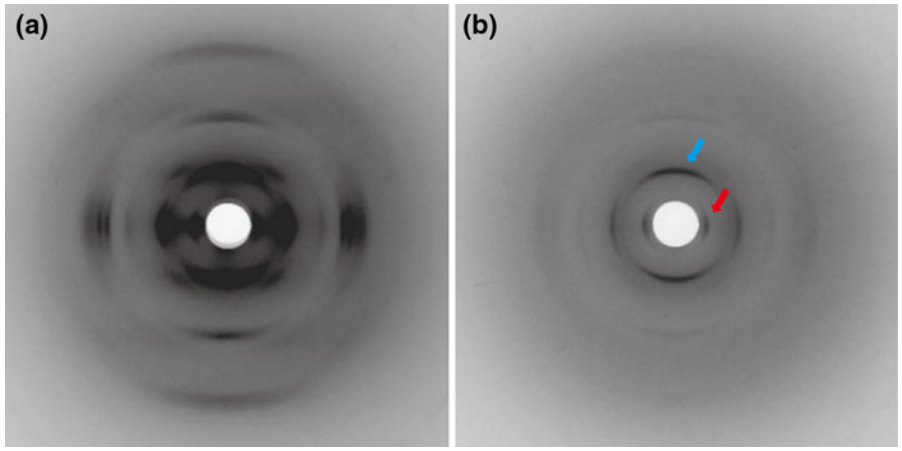


Fig. 4 X-ray fiber diffraction patterns of β-chitin succinate treated with aq. $Pb(CH_3COO)_2$. **a** Regular exposure and **b** weak exposure for showing strongest reflections only. *Red* and *blue arrows* show the sheet spacing of 1.69 nm and the fiber repeat of 1.03 nm, respectively

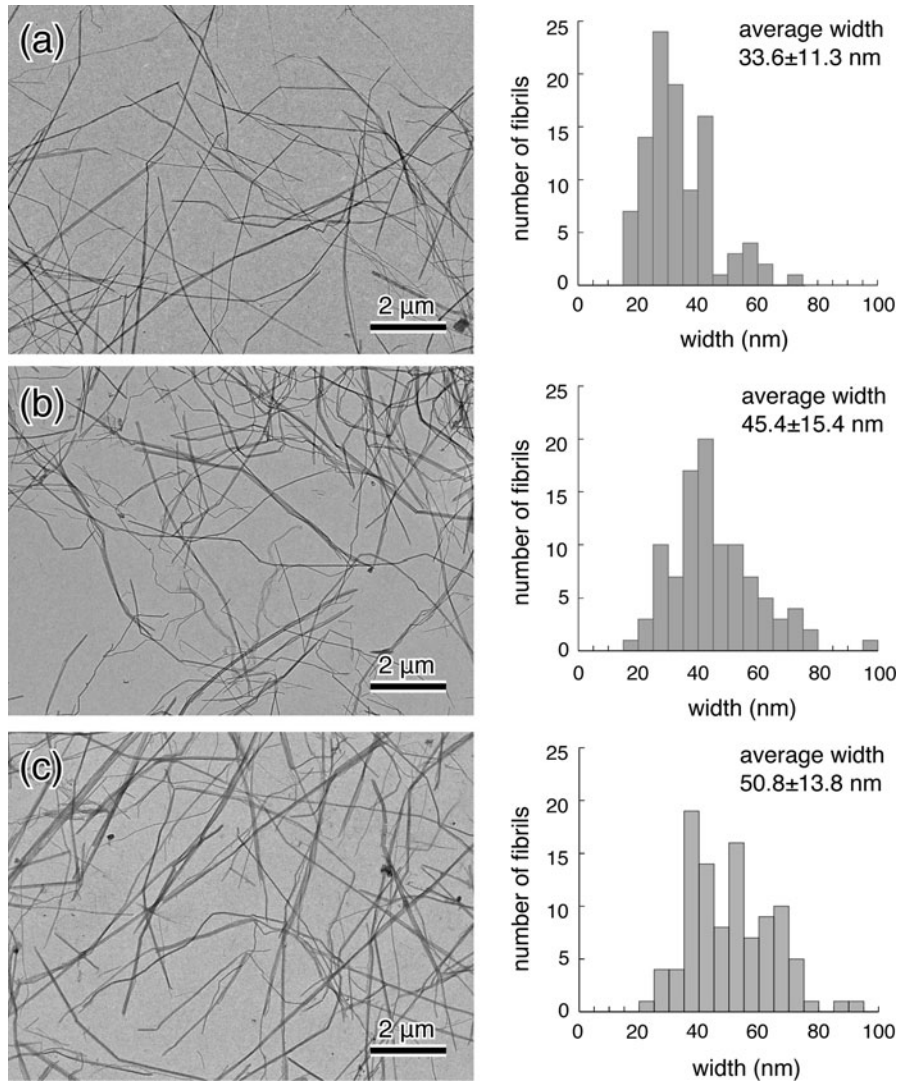


Fig. 5 Bright field diffraction contrast images of **a** initial β-chitin and its succinate **b** before and **c** after aq. Pb(II) acetate treatment, and their width distributions

order and microfibrillar morphology. Such characteristic regular arrangements of metal cations in the biopolymer crystal may find utility as novel nanomaterials.

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References

Blackwell J (1969) Structure of β-chitin or parallel chain systems of Poly-β-(1 → 4)-N-acetyl-D-Glucosamine. Biopolymers 7:281–298

Dweltz NE, Colvin JR, McInnes AG (1968) Studies on chitoan (β-(1 → 4)-linked 2-acetoamido-2-deoxy-D-glucan) fibers of the diatom *Thalassiosira fluviatilis*, Hustedt. III. The structure of chitan from X-ray diffraction and electron microscope observations. Can J Chem 46:1514–1521

Nishiyama Y, Noishiki Y, Wada M (2011) X-ray structure of anhydrous β-chitin at 1 Å resolution. Macromolecules 44:950–957

Noishiki Y, Nishiyama Y, Wada M, Okada S, Kuga S (2003) Inclusion complex of β-chitin and aliphatic amines. Biomacromolecules 4:944–949

Noishiki Y, Kuga S, Wada M, Hori K, Nishiyama Y (2004) Guest selectivity in complexation of β-chitin. Macromolecules 37:6839–6842

Ogawa Y, Kimura S, Wada M (2011) Electron diffraction and high-resolution imaging on highly-crystalline β-chitin microfibril. J Struct Biol 176:83–90. doi:10.1016/j.jsb.2011.07.001

Saito Y, Okano T, Putaux J-L, Gaill F, Chanzy H (1998) Crystallosolvates of β-chitin and alcohols. In: Domard EA,

Roberts G, Vårum K (eds) Advances in chitin science II. Jacques André Publishers, Lyon, pp 507–512

Saito Y, Tomotake Y, Shida S (2007) Formation of a lamellar compound by reaction of acrylic acid crystallosolvated in highly crystalline β-chitin. Biomacromolecules 8:1064–1068

Sawada D, Nishiyama Y, Langan P, Forsyth VT, Kimura S, Wada M (2012) Water in crystalline fibers of dihydrate β-chitin results in unexpected absence of intramolecular hydrogen bonding. PLoS ONE 7:e39376. doi:10.1371/journal.pone.0039376

Sawada D, Kimura S, Nishiyama Y, Langan P, Wada M (2013) The crystal structure of mono-ethylenediamine β-chitin from synchrotron X-ray fiber diffraction. Carbohydr Polym 92:1737–1742. doi:10.1016/j.carbpol.2012.11.025

Yoshifuji A, Noishiki Y, Wada M, Heux L, Kuga S (2006) Esterification of β-chitin via intercalation by carboxylic anhydrides. Biomacromolecules 7:2878–2881. doi:10.1021/bm060516w