Articles

Formation of a Lamellar Compound by Reaction of Acrylic Acid Crystallosolvated in Highly Crystalline β-Chitin

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A lamellar compound resulted from reaction of acrylic acid inside crystalline β-chitin and the structure was investigated. β-Chitin acts like a layered crystal, having stacked molecular sheets composed of parallel chains bound in one direction by intermolecular amide hydrogen bonding. Small guest molecules can be inserted between the molecular sheets, and a crystallosolvate can be formed. By immersion of β-chitin in acrylic acid, a crystallosolvate was formed, which was then changed into the more stable lamellar compound by heat treatment at 105 °C. NMR measurement and IR spectroscopy showed that during the heat treatment there was a reaction between acrylic acid and the β-chitin molecular sheet, but the sheet structure was maintained. By IR with deuteration, it was shown that the accessibility of solvents to this lamellar compound was greater than that for the initial β-chitin. The lamellar compound is considered a kind of "pillared" structure related to the lamellar crystal.

Introduction

Chitin, poly[β(1→4)-2-acetamido-2-deoxy-D-glucopyranose], is the most abundant structural polymer, next to cellulose, on the Earth. Chitin is a component of fungi, insects, and crustaceans. It is commonly found in the form of crystals corresponding to two allomorphs, namely α and β.[1] α-Chitin is the most abundant, occurring in the cuticle of insects, crabs, and shrimps, in the exoskeleton of krill and other organisms. Crystallographically, it has $P2_12_12_1$ symmetry,[2] which means the unit cell of α-chitin contains two antiparallel polymer chains. β-Chitin is rather rare, found in squid pens, the spines of some diatoms, the tubes of pogonophores and vestimentiferans, and others. In the β-chitin crystal, chains are arranged parallel in the same direction, having $P2_1$ symmetry with only one chain per unit cell.

β-Chitin has the property of forming crystallosolvates very easily,[3] as opposed to α-chitin, which is thermodynamically quite stable and scarcely forms crystallosolvates, except when treated with some amines.[4] β-Chitin hydrates[5] are the best-known examples of crystallosolvates, where small solvent molecules are easily accepted within the β-chitin lattice. The crystallosolvate easily reverts to anhydrous β-chitin upon drying, without any apparent modification of morphology and crystallinity from the parent anhydrous β-chitin. By infrared spectroscopy (IR) with deuteration, it is shown that intermolecular amide hydrogen bonds are retained throughout crystallosolvation.[6] As long as amide hydrogen bonds are maintained, neighboring chains are arranged as a molecular sheet in the *a*-direction of the β-chitin unit cell, behaving like a kind of "lamellar crystal". Thus, crystallosolvation of β-chitin involves insertion of guest molecules between stacked chitin molecular sheets, occurring like "intercalation" with layer crystals, as with graphite or some mineral crystals.

The lamellar crystal-like property of β-chitin is expected to have many applications in the future because of the huge crystal surface area. A crystalline lamellar surface is useful in the field of chemical reactions with special chirality, or when a huge absorption area is required. However, not each surface of the lamellar crystal layers is useful because the layers are closely packed and the surfaces are inaccessible. However, β-chitin has an intercalation-like property. Intercalation is a phenomenon that is generally observed for ion-exchange-lamellar crystals because the layers need to be electrically charged to introduce guest molecules. β-Chitin is unique because of easy insertion of guest molecules between the molecular sheets, though it is a type of molecular-lamellar crystal, without ionic properties which sometimes induces the intercalation process.

To utilize effectively the layer crystal surface, molecular-sized "pillars" put between each layer are useful. This pillaring technique may result in selective absorption ability, which some clay minerals have. In this study, we tried to insert pillars between the β chitin molecular sheets. For this to happen, the molecular sheet structure should be retained, the pillars should be stable between the sheets, and the distribution of the pillars should be dispersed rather than dense. If pillaring for β-chitin is possible, the huge area of the crystalline surface of β chitin is usable, which is applicable for absorbent or molecular sieves. Acrylic acid was introduced between the β-chitin molecular sheets and it reacted there. The structure of the resulting compound was investigated using X-ray diffraction, transmission electron microscopy (TEM), nuclear magnetic resonance (NMR), and infrared spectroscopy (IR).

Experimental Section

Specimens. Tubes from *Lamellibrachia* sp. were deproteinized as described elsewhere.[7] The purified tubes consisted of highly crystalline β-chitin microfibrils[7] that were delaminated and formed as a sheet by drying on Teflon plates.

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Specimen Crystallosolvation and Reaction. The purified *Lamellibrachia* sample was immersed in water, and extra water was removed by squeezing between filter papers. Then the sample was immersed in acrylic acid. For the X-ray diffraction measurement, it was immediately sealed in a 1 mm diameter glass capillary. For reaction with acrylic acid, the sample inside the capillary with one end closed and the other end open was placed in an oven at 105 °C. For the NMR measurement, the sample was sandwiched between two glass plates and heated for 9 h in an oven at 105 °C. After the heat treatment, extra acrylic acid was removed, and the homogeneous reaction of the sample into the lamellar compound was verified by X-ray diffraction.

X-ray Diffraction Analysis. The specimens were X-rayed with Ni-filtered Cu Kα radiation using a Rigaku X-ray generator operated at 50 kV and 100 mA with a vacuum camera. The diffraction patterns were recorded on Fuji imaging plates. Spacings of reflections on X-ray patterns were calibrated using that of NaF as standard.

Transmission Electron Microscopy. The specimen was delaminated by using tweezers and mounted on an electron microscope grid. Electron microscopy observation with bright field contrast and selected area diffraction were achieved with a JEOL 2000EX.TEM operated at 200 kV under low-dose conditions.

NMR Spectroscopy. The solid-state ^{13}C NMR measurement was carried out with a Bruker MSL-300, operating at a carbon frequency of 75.46 MHz. Spectra were acquired with cross-polarization magic-angle spinning (CPMAS) techniques with a spinning rate of 5 kHz for the intact β-chitin sample and 4.2 kHz for the acrylic acid immersed and reacted samples, in a 7.5 mm internal diameter probe head. The contact time was 1 ms, and the pulse delay between scans was 10 s. To detect the liquid fraction of the sample immersed in acrylic acid, the dipole−dipole/magic angle spinning (DDMAS) method with a wideband alternating-phase low-power technique for zero-residual splitting (WALTZ) was used for ^{1}H decoupling. By this method, the intensity of the ^{1}H decoupling was weakened as in a liquid NMR measurement, and ^{13}C was measured directly without CPMAS but with a short delay of 5 s. All spectra were recorded at room temperature.

FTIR Measurement. The infrared measurements were performed on a thin film sample. Prior to FTIR analysis, all specimens were dried completely at 105 °C overnight to remove all traces of solvent and moisture. They were verified to be in the anhydrous state by X-ray diffraction. The sample was analyzed using a Nicolet Magna 860 FTIR spectrometer under a flux of dried air. For measurement of deuterated sample with liquid D_2O, the dried samples were then immersed in D_2O for 2 h and finally dried at 105 °C overnight and measured. For deuteration by D_2O vapor, an airtight cell was prepared, a small vessel of liquid D_2O was placed inside the cell, and the cell was evacuated. Then the cell was sealed, and after the inside of the cell was saturated with D_2O vapor, the IR measurement was performed in situ. All spectra were recorded in transmission mode with a resolution of 2 cm^{-1}.

Results and Discussion

X-ray Analysis. In Figure 1, a series of X-ray diagrams of a *Lamellibrachia* β-chitin sample corresponding to each step of the procedure is shown. The 5−10° region is the part where the peak indicating the distance between adjacent β-chitin molecular sheets appears. The initial spectrum is that of highly crystalline anhydrous β-chitin, with a peak at 9.54°, corresponding to the sheet distance of 0.927 nm (Figure 1a). When the sample was immersed in water, the peak shifted to a lower angle, at 7.61°, with a sheet separation of 1.16 nm (Figure 1b), which corresponds to hydrated β-chitin.[5] Following immersion in acrylic acid, the peak shifted to a much lower angle, 6.27°, with a sheet separation of 1.41 nm (Figure 1c). This change suggests that a crystallosolvate of β-chitin with acrylic acid was formed, by insertion of acrylic acid molecules between the β-chitin molecular sheets. When the sample immersed in acrylic acid was heated at 105 °C, the peak gradually shifted to a higher angle (X-ray diagram not shown) and, after 15 days of heat treatment, it became almost stable and constantly observed at 7.04°, with a sheet separation of 1.255 nm (Figure 1d). Although several washings with water reduced the sheet separation to 1.15 nm (Figure 1f), the sample after heat treatment with acrylic acid seemed to be very stable and repeated washing with water made the molecular sheet separation more stable (approximately 1.15 nm). The sheet separation, 1.15 nm, was much larger than that of anhydrous β-chitin. This fact suggests that acrylic acid was kept between the sheets after the heat treatment. In contrast, before heat treatment the crystallosolvate with acrylic acid reverted to anhydrous β-chitin easily on repeated washing with water. Moreover, when the sample after heat treatment with acrylic acid was immersed in water, the molecular sheet separation was approximately 1.30 nm (Figures 1e and 1g), and the space of sheets was much larger than that for anhydrous β-chitin (Figure 1a). These facts indicate that, under heat treatment, acrylic acid had some kind of interaction between the β-chitin molecular sheets and the sheet separation was fixed larger than that in the initial anhydrous β-chitin.

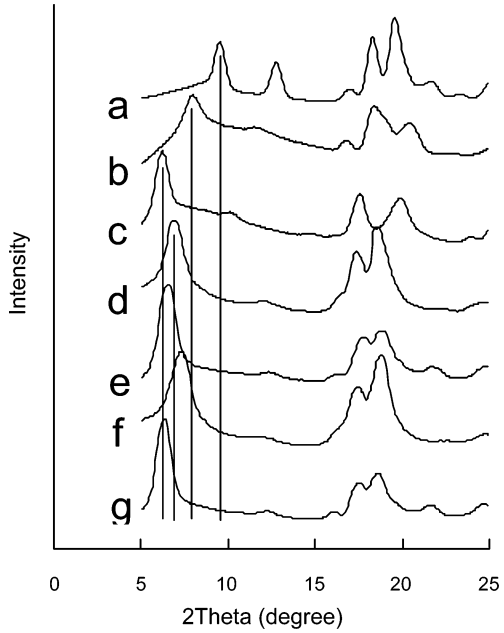

Figure 1. X-ray diffractograms of *Lamellibrachia* β-chitin during the sequential procedure: (a) initial sample oven-dried; (b) impregnated with water; (c) impregnated with acrylic acid; (d) heat-treated for 15 days; (e) impregnated with water; (f) dried overnight; (g) impregnated with water.

Figure 2 shows a TEM micrograph of the β-chitin after heat treatment with acrylic acid, corresponding to the state as in Figure 1d. The electron diffractogram indicates that the molecular sheet separation was kept even under the highly evacuated conditions of TEM observation, whereas without heat treatment the crystallosolvate with acrylic acid reverted to anhydrous β-chitin easily upon removing the acrylic acid by evacuation. The fibrous feature of β-chitin microfibril has been observed, as in intact β-chitin, which implies that the parallel arrangement of the chitin chain molecules is maintained even after the heat treatment with acrylic acid.

NMR Measurement. NMR measurements were performed on each stage of the lamellar compound formation of β-chitin and acrylic acid (Figure 3 and Table 1). The intact *Lamellibrachia* sample shows the spectrum of a typical highly crystalline β-chitin[8] (Figure 3a). After impregnation in acrylic acid, liquid acrylic acid was observed by the DDMAS method (Figure

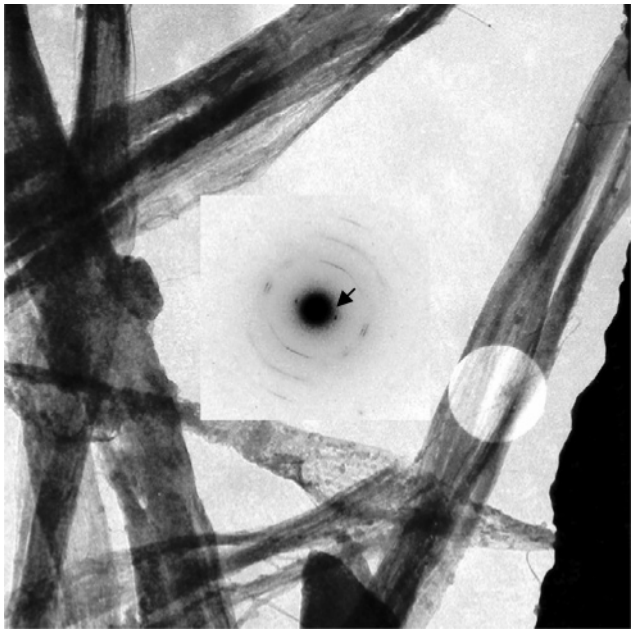

Figure 2. Transmission electron micrograph by diffraction contrast of *Lamellibrachia* β-chitin after heat treatment with acrylic acid. In the middle, a selected area of diffraction from the circled area of 1 μm diameter on the image is shown. Arrow: reflection corresponding to 1.14 nm, which indicates an increased distance between chitin molecular sheets.

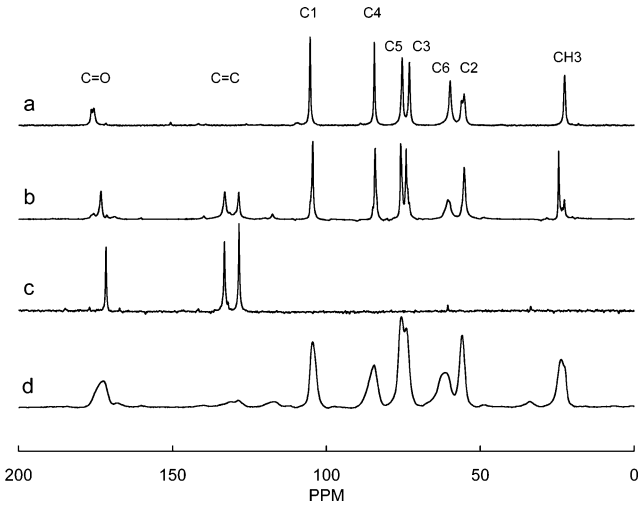

Figure 3. ^{13}C NMR spectra of *Lamellibrachia* β-chitin: (a) initial sample; (b) and (c) impregnated with acrylic acid; (d) after heat treatment with acrylic acid. (a), (b), and (d) are solid-state ^{13}C CPMAS and (c) is DDMAS for liquid detection.

3c), whereas solid was selectively observed by CPMAS (Figure 3b), although the C═C spectra originating from acrylic acid in CP/MAS are slightly broader (Figure 3b) than those in DD/MAS (Figure 3c). This fact implies that the molecules of acrylic acid are arranged in an orderly manner between the sheets of β-chitin. On the other hand, in the C═O region, three additional peaks were observed compared with the intact β-chitin. This splitting is consistent with the fact that the CH_3 peaks are also split into three, probably due to CH_3 of β-chitin linked to C═O. The higher region shift of CH_3 (22 to 24 ppm) shows a similar tendency to that in hydration of β-chitin.[8] For C6 of β-chitin at 60 ppm, the peak width is broadened with the crystallosolvate, compared with the anhydrous β-chitin. This change around C6 with β-chitin may be due to the newly formed interaction between O6H and −COOH of acrylic acid. In conclusion, under impregnation in acrylic acid, the β chitin forms crystallosolvate, where acrylic acid exists as the solid phase: The acrylic acid is placed between the chitin molecular sheets and −COOH of acrylic acid probably interacting with C6OH of β-chitin. But splitting of C═O of the β-chitin suggests that the crystallosolvate is in several different states.

After heat treatment of the β-chitin crystallosolvate, the spectrum became broader (Figure 3d). This means that the degree of crystallinity diminished, although it still has some crystalline ordering, as shown in Figures 1d and 1f. Because a new peak appeared at 34 ppm and the peaks at 128 and 133 ppm diminished, acrylic acid in the crystallosolvate should be polymerized in part. Mostly, polymerization of acrylic acid occurs at higher temperature with the help of a catalyst. However, in this case it polymerized at low temperature and without any catalyst. This might be because the reaction happened in a different way affected by the chitin molecular sheets. The asymmetrical shape of the C═O peak around 172 ppm indicates that several peaks overlap. Broad peaks were also observed at 180−190 ppm, but these are difficult to assign; they may be spinning side band peaks originating from acrylic acid. Using the published data,[9] 172, 176, and 168 ppm could be assigned as C═O from chitin, ester of poly(acrylic acid) or hydrogen-bonded poly(acrylic acid), and hydrogen-bonded acrylic acid or ester of poly(acrylic acid), respectively. Following heat treatment, the peaks of C6 shifted to higher ppm, compared with the crystallosolvate, although those of C3 did not. This indicates that O6H had a stronger interaction with acrylic acid than in the crystallosolvate, similar to ester bonding with acrylic acid, whereas O3H was not affected by the heat treatment. In conclusion, heat treatment partially polymerized acrylic acid between the chitin molecular sheets, and acrylic acid molecule or poly(acrylic acid) molecules produced some kind of interaction, like hydrogen bonding or covalent bonding, between O6H of chitin. This fact indicates that even after heat treatment, the parallel arrangement of chitin chain is maintained, owing to intermolecular hydrogen bonding of O3H...O5, which keeps the straightness of the chitin chain. In the following part, we call the stacked sandwich-like structure of the resultant product after heat treatment of the crystallosolvate a "lamellar compound".

IR Spectroscopy with Deuteration. IR spectroscopy of the lamellar compound was performed (Figure 4). In comparison to the intact β-chitin spectrum (Figure 4a), that of the lamellar compound is broader, but the peaks appear in similar positions generally, except for the peak at 1700−1800 cm^{-1}, where C═O of acrylic acid should appear (Figure 4b). Further investigation of the area from 1400 to 1800 cm^{-1} was achieved, where anhydrous β-chitin has single peaks at 1630 cm^{-1} due to C═O (Figure 4a), which has branched intermolecular hydrogen bonds between N−H and O6H of the neighboring chitin chain. With the lamellar compound, an additional peak at 1653 cm^{-1} appeared (Figure 4b), which is in a very similar position to the case when C═O is hydrogen-bonded singly to H−N, without branching.[6] By the heat treatment with acrylic acid, the conformation of C6OH was changed and the hydrogen bonding of C═O...H6O was broken and the C═O hydrogen bonding became single. This change is consistent with the result of NMR measurements, showing C6 shift. By formation of the lamellar compound, the NH stretching band at 3293 cm^{-1} became broader and shifted slightly to lower wavenumber. This is possibly due to an increase of strength of intermolecular hydrogen bonding by cleavage between C═O...H6O, or newly formed amide hydrogen bonds between C═O of acrylic acid. N−H at 1560 cm^{-1} was almost unaffected by formation of the lamellar compound. It is considered intermolecular amide

Table 1. ^{13}C Chemical Shifts of *Lamellibrachia* β-Chitin of Initial, Impregnated with Acrylic Acid, and after Heat Treatment with Acrylic Acid

			impregnated with acrylic acid		after heat treatment
		initial	CP/MAS	DD/MAS	with acrylic acid
chitin	C1	105.3	104.4		104.4
	C2	55.2	55.1		55.8
		56.0			
	C3	73.0	74.0		73.8
	C4	84.3	84.1		84.4
	C5	75.3	75.8		75.4
	C6	59.7	59.9		60.5
			60.6		62.5
	CH_3	22.5	22.7		23.7
			23.3		
			24.5		
chitin or acrylic acid	C═O		168.7		167.9
			171.3	171.5	172.4
			173.1		
		175.5	175.5		
		176.3	176.2		
acrylic acid	C═C		128.5	128.4	128.6
			133.1	133.1	
polymeric	C−C				33.8

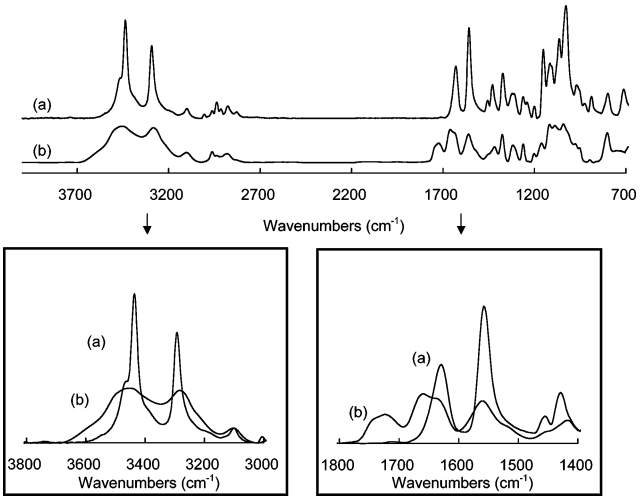

Figure 4. FTIR spectra of *Lamellibrachia* β-chitin: (a) initial sample; (b) after heat treatment with acrylic acid.

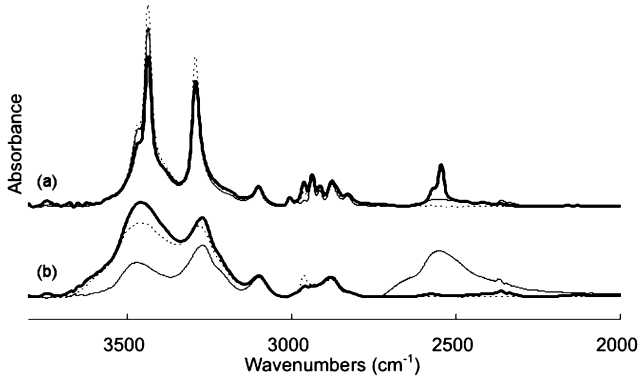

Figure 5. FTIR spectra of *Lamellibrachia* β-chitin: (a) initial sample; (b) after heat treatment with acrylic acid. Bold line: deuterated in liquid D_2O and dried before measurement; straight line: in situ measurement in D_2O vapor; dotted line: no deuteration.

hydrogen bonds still exist in the crystallosolvate, maintaining the molecular sheet structure of β-chitin.[6]

It is known that chitosan (degree of deacetyration >60%) in acrylic acid water solution changes into *N*-carboxyl-ethyl-chitosan by the Michael reaction.[10] However, in our case, the situation is different because it is not in water solution; moreover, acetyl amide groups were blocked because of strong amide-hydrogen bonding. Thus, the Michael addition reaction is considered not to occur in our case.

Deuteration IR Spectroscopy. Accessibility of water to the lamellar compound was investigated (Figure 5), which might give a clue to elucidate the structure of the lamellar compound. Liquid D_2O and vaporized D_2O were used. The appearance of deuterated peaks due to OD was expected in the region of 2300−2700 cm^{-1}, if the OH groups in the lamellar compound have interaction with the D_2O molecules. In the initial β-chitin, OD peaks around 2500−2600 cm^{-1}[6] were only observed after treatment with liquid D_2O (Figure 5a). In the case of initial β-chitin, because of high crystallinity, vaporized D_2O cannot access inside, but liquid D_2O can. On the other hand, in the lamellar compound, the OD peaks were only observed in situ with vaporized D_2O (Figure. 5b). It is considered that the molecular sheets of the lamellar compound are not so tightly packed that even vaporized D_2O can access inside. Because of this high accessibility, once substituted OD can be easily substituted by OH in air; thus, the OD peak could be observed only in situ with vaporized D_2O. It is concluded that the lamellar compound has a much more accessible chitin molecular sheet, compared with the intact β-chitin. The prior accessibility of the lamellar compound is also shown by the following fact: A solvent like butanol, propanol, pentanol, decanol, ethylenglycol, 1,3-butanediol, 1,4-butanediol, 1,5-pentanediol, and 1,5-hexanediol can be inserted into the lamellar compound without any pretreatment and easily form the crystallosolvate with the lamellar compound by direct immersion. These solvents are never to be inserted into intact β-chitin by just immersing, without pretreatment with immersion in water or diluted hydrochloric acid.

Figure 6 shows schematically the changes in crystallosolvation and "pillaring" of β-chitin. The lamellar compound consists of two components: one is a chitin molecular sheet bound by amido hydrogen molecules and the other is a pillar of acrylic acid which is partially polymerized or bonded to chitin. Based on elemental analysis by using CHN coder, the lamellar compound consists of 45.83 wt % C, 6.18 wt % H, and 5.59 wt % N: If any deacetylation reaction is neglected, one molecule

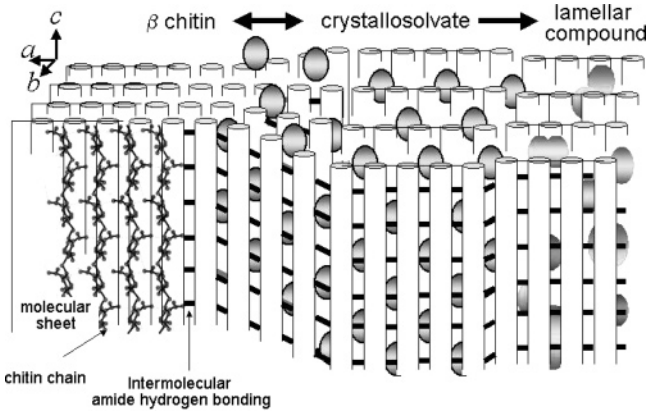

Figure 6. Schematic drawing of chitin crystallosolvates and lamellar compound.

of acrylic acid is inserted per two *N*-acetyl glucosamine residues of β-chitin. The pillars are not dense but dispersed and weaken the aggregation force of the sheets and are accessible for other solvent molecules.

Conclusion

Acrylic acid can be inserted between the β-chitin molecular sheets and the crystallosolvate could be formed by immersion of β-chitin in acrylic acid, by immersing the dried chitin in water. The crystallosolvate changes into the more stable lamellar compound by heat treatment at 105 °C, by reaction of acrylic acid itself, and between acrylic acid and the β-chitin molecular sheets. The accessibility of solvents to this lamellar compound was greater than that of the initial β-chitin.

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References and Notes

(1) Rudall, K. M.; Kenchington, W. *Biol. Rev.* **1973**, *49*, 597−636.
(2) Minke, R.; Blackwell, J. *J. Mol. Biol.* **1978**, *120*, 167−181.
(3) Saito, Y.; Okano, T.; Putaux, J. L.; Gaill, F.; Chanzy, H. In *Advances in Chitin Science, Seventh ICCC, vol. II. Lyon, France*; Domard, A., Roberts, G. A. F., Vårum, K. M., Eds.; Jacque André Publisher: 1997; pp 507−512.
(4) Noishiki, Y.; Nishiyama, Y.; Wada, M.; Kuga, S. *Biomacromolecules* **2005**, *6*, 2362−2364.
(5) Blackwell, J. *Biopolymers* **1969**, *7*, 281−298.
(6) Saito, Y.; Okano, T.; Gaill, F.; Chanzy, H.; Putaux, J. L. *Int. J. Biol. Macromol.* **2000**, *28*, 81−88.
(7) Gaill, F.; Persson, J.; Sugiyama, J.; Vuong, R.; Chanzy, H. *J. Struct. Biol.* **1992**, *109*, 116−128.
(8) Tanner, F.; Chanzy, H.; Vincendon, M.; Roux, J. C.; Gaill, F. *Macromolecules* **1990**, *23*, 3576−3583.
(9) Lau, C.; Mi, Y. *Polymer* **2002**, *43*, 823−829.
(10) Sashiwa, H.; Yamamori, N.; Ichinose, Y.; Sunamoto, J.; Aiba, S. *Macromol. Biosci.* **2003**, *3*, 231−233.

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