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ORIGINAL PAPER

Preparation and characteristics of reprecipitated chitin: a new morphological form easy to manipulate with versatile utility

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Abstract With the object of effectively destroying the rigid crystalline structure of chitin and thereby preparing an amorphous form with enhanced reactivity, chitin was dissolved in methanol saturated with calcium chloride dihydrate and reprecipitated under appropriate conditions. Chitin in the solution was first precipitated in deionized water, and the mixture was subjected to dialysis followed by freeze-drying. As an alternative method, the chitin solution was directly dialyzed and freeze-dried. Judging from the appearances of the products, the latter method was much superior, giving rise to the formation of highly fluffy cotton-like chitin. Compared to β-chitin, α-chitin was suitable as a starting material because of the adequate solubility in the solvent system. The resulting reprecipitated chitin exhibited higher reactivity as confirmed by the adsorption of copper(II) and acetylation reaction. The method was quite simple and convenient to prepare reprecipitated chitin that will have high potential as a new form from the practical viewpoint.

Keywords Chitin · Reprecipitation · Amorphous chitin · Copper adsorption · Chemical modifications · Acetylation · Methanol/calcium chloride

Introduction

Chitin, an amino polysaccharide, is an abundant natural polymeric material occurring in many species and accessible easily particularly from crab and shrimp shells in food waste. As the distinctive characteristics of chitin are being disclosed

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including certain biological and medicinal activities, high adsorption capacity, and film- and fiber-forming properties, it has become one of the most interesting biopolymers attracting much attention in a wide variety of fields [1–8]. However, chitin remains an almost unutilized biomass resource primarily because of the difficulty in fabrication and chemical modifications owing to the poor affinity to solvents. It is not soluble in solvents except some special ones including fluorinated compounds such as hexafluoroacetone and hexafluoro-2-propanol [9], *N*,*N*-dimethylacetamide and/or *N*-methylpyrrolidone containing lithium chloride [9, 10], and methanol/calcium chloride [11]. The strong intermolecular forces are responsible for the inherent crystallinity and thus limited solubility, which has undoubtedly delayed the basic and utilization studies of this unique biopolymer with high potential.

A clue to overcoming the intractable nature of chitin may be effective destruction of the crystalline structure, and reprecipitation under appropriate conditions would be promising as suggested by the formation of amorphous chitosan reported in our previous article [12]. In order to destroy the rigid crystalline structure of chitin and consequently to prepare a new morphological form easy to manipulate, some factors of reprecipitation and isolation have been studies. We report here the preparation of reprecipitated chitin and the improved reactivity as compared with original chitin.

Experimental

General

IR spectra were recorded on a Shimadzu FTIR-8900 instrument by the KBr method. UV–vis spectra were taken with a JASCO V-530 spectrophotometer. X-ray diffraction diagrams were obtained by the powder method with the use of Ni-filtered Cu Kα radiation with a MAC Science M03X-HF 1013. Elemental analysis was performed on a Perkin Elmer 2400 II instrument. The conductometric titration was carried out with a DKK TOA conductivity meter CM-20J to determine the extent of *N*-acetylation [13]. Solvents were purified in usual manners and stored over molecular sieves. All the chemicals were of reagent grade and used without further purification.

Chitin

α-Chitin was isolated from shrimp shells [14], and the degree of deacetylation was around 0.1. It was treated with acetic anhydride in methanol to acetylate the free amino groups by the method described previously [15]. In brief, 3.00 g of pulverized α-chitin was suspended in 200 mL of methanol, and 100 mL of acetic anhydride was added. After 48 h at 40 °C, the product was isolated in ice water and treated with 0.1 M potassium hydroxide in methanol at room temperature for 5 h to give 2.70 g (90%) of fully *N*-acetylated chitin as confirmed by conductometric titration. IR (KBr): ν 1655 (amide I), 1558 (amide II), and 1150–1000 cm^{-1} (pyranose).

β-Chitin isolated from squid pens [16] was kindly provided by Toyo Suisan Kaisha, Ltd. and similarly *N*-acetylated in a yield of 89%.

Reprecipitation of chitin

α-Chitin was thoroughly dried with phosphorus pentoxide, and 0.200 g of the sample was added to 100 mL of methanol saturated with calcium chloride dihydrate. The mixture was stirred at room temperature overnight and centrifuged to remove remaining some swollen gels. The supernatant was dialyzed in deionized water for 2 days to form a cloudy precipitate of chitin. The resulting mixture was freeze-dried and then dried in vacuum at 60 °C to give 0.122 g (61%) of chitin as a white fluffy cotton-like material. IR (KBr): ν 1651 (amide I), 1558 (amide II), and 1150–1000 cm^{-1} (pyranose).

In another run, a mixture of β-chitin in the same solvent was refluxed for 5 h because of the difficulty in dissolution, and the solution containing swollen gels was poured into deionized water. The mixture was dialyzed and freeze-dried to give the reprecipitated product in a yield of 89%.

Adsorption of copper(II)

Reprecipitated or pulverized original α-chitin, 10 mg, was added to 25.0 mL of 0.5 mmol/L aqueous copper(II) chloride solution, and the mixture was stirred at room temperature for a prescribed time. It was filtered with a glass filter, and the copper content in the filtrate was determined by UV–vis spectroscopy using diethyldithiocarbamic acid by the method reported elsewhere [17].

Acetylation

To 10 mL of pyridine containing 45 mg (0.37 mmol) of 4-dimethylaminopyridine (DMAP) were added 50 mg (0.246 mmol) of reprecipitated α-chitin and 2.51 g (24.6 mmol) of acetic anhydride. The mixture was stirred at room temperature for 72 h in a nitrogen atmosphere and poured into ice water. The precipitate was collected by centrifugation, washed with deionized water (50 mL 3×) and with acetone (50 mL 1×), and dried to give 34 mg of the product. The degree of substitution (ds) for the *O*-acetyl group was determined to be 0.88 from the C/N ratio of elemental analysis. The yield was calculated to be 58% on the basis of the ds value. IR (KBr): ν 1735 (ester C=O), 1658 (amide I), 1558 (amide II), and 1150–1000 cm^{-1} (pyranose).

Anal. Calcd for $(C_{10}H_{15}NO_6)_{0.88}(C_8H_{13}NO_5)_{0.12}\cdot0.4H_2O$: C, 47.39; H, 6.34; N, 5.66. Found: C, 47.34; H, 6.15; N, 5.71.

Under the same reaction conditions, 50 mg of pulverized original α-chitin gave 27 mg of the acetylated product, whose ds for *O*-acetyl was 0.38. The yield was 50% based on the ds value.

Anal. Calcd for $(C_{10}H_{15}NO_6)_{0.38}(C_8H_{13}NO_5)_{0.62}\cdot0.6H_2O$: C, 45.75; H, 6.56; N, 6.09. Found: C, 45.59; H, 6.41; N, 6.07.

Results and discussion

Reprecipitation of chitin

In order to discuss the physicochemical properties of the reprecipitated chitin, it is crucial to use structurally well-defined chitin as a starting material, and fully *N*-acetylated chitin was prepared beforehand by selective *N*-acetylation of α-chitin isolated from shrimp shells. The resulting chitin of a degree of *N*-acetylation 1.0 was dissolved in methanol saturated with calcium chloride dihydrate, a convenient and easy to handle solvent system [11]. The solution was poured into deionized water to precipitate chitin. The mixture was subsequently dialyzed in water and freeze-dried to give a white fluffy solid.

In an alternative way, the chitin solution was dialyzed directly to slowly precipitate chitin, and the resulting cloudy mixture was freeze-dried to give a product that was much more voluminous and fluffy than that by the former method, indicating the latter method to be superior. Figure 1 compares the appearances of the reprecipitated α-chitin by the latter method and original α-chitin of the same weight (0.1 g). The X-ray diffraction diagram of the reprecipitated chitin showed no distinct peaks.

In a similar manner, *β*-chitin could also be reprecipitated, but α-chitin was found more suitable to prepare reprecipitated chitin in view of the solubility in methanol/calcium chloride. Compared to α-chitin, *β*-chitin was difficult to dissolve in the solvent [11], and the limited solubility was not improved even at elevated temperatures, resulting in the formation of a considerable amount of gels. Reprecipitated α-chitin was thus used to examine the influence of reprecipitation on some properties of chitin.

Adsorption of copper(II)

As a typical example of metal collection ability, reprecipitated and pulverized original α-chitins were treated with aqueous copper(II) under heterogeneous

Fig. 1 Original α-chitin (*left*) and reprecipitated α-chitin (*right*) of the same weight prepared by dissolution in methanol/calcium chloride followed by dialysis and freeze-drying

conditions, and the amount of cupper(II) left in the supernatant was determined by spectrometry. As shown in Fig. 2, adsorption by reprecipitated chitin was more facile, especially in the early stage, but the difference became small on prolonged treatment. This was interpreted in terms of the difference in the swelling ability and surface area of the two kinds of chitin samples.

Acetylation

Reprecipitation of α-chitin was expected to improve chemical reactivity as in the case of reprecipitation of chitosan [12], and acetylation was carried out with acetic anhydride in pyridine in heterogeneous suspension under various conditions (Scheme 1). The results are shown in Fig. 3. As evident in the figure, the reprecipitated chitin (solid symbols) exhibited much higher reactivity than the pulverized original chitin (open symbols). Swelling in pyridine for 24 h before the reaction was not very effective for the original chitin (see open squares and triangles), but it was remarkable for the reprecipitated chitin (see solid squares and triangles). The addition of DMAP confirmed to facilitate acetylation to a considerable extent (see open and solid triangles and circles). For instance, the reprecipitated chitin resulted in the formation of acetylated chitin with a ds 0.88 for *O*-acetyl in 72 h reaction in the presence of DMAP (solid circle), while the ds was as low as 0.38 with the original chitin under the same reaction conditions (open circle).

The above results implied that the swelling would have played an important role to facilitate the acetylation reaction, and the influence of prior swelling in pyridine

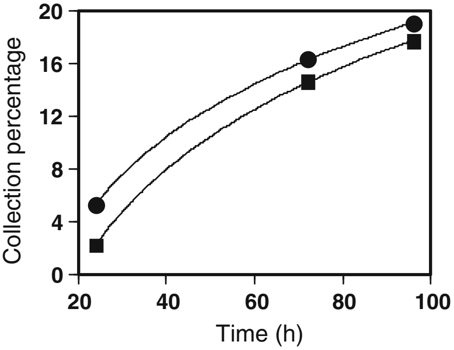
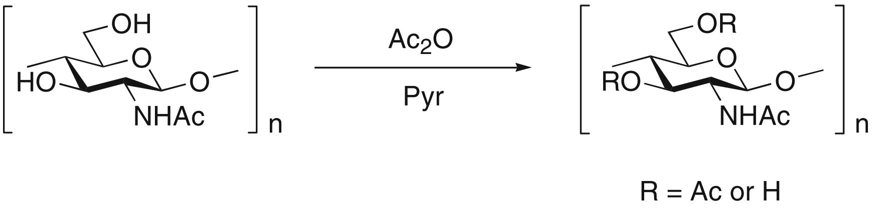
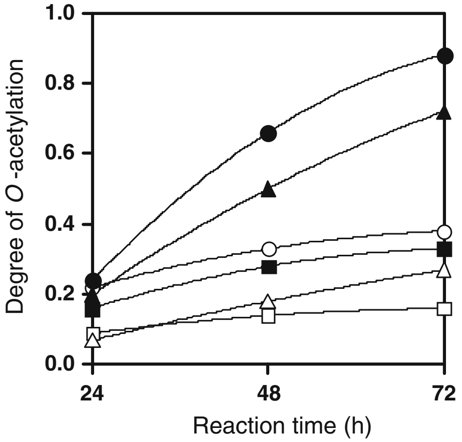


Fig. 2 Adsorption of copper(II) by reprecipitated α-chitin (*solid circle*) and original α-chitin (*solid square*)



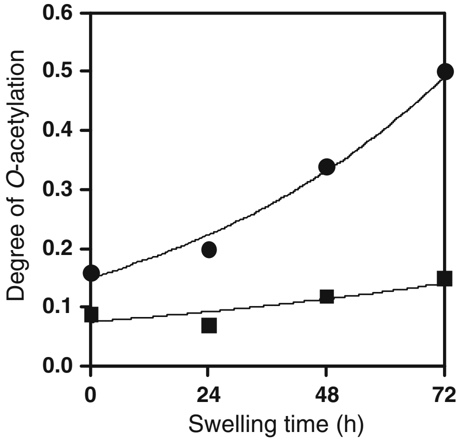
Scheme 1 Acetylation of chitin



symbol	chitin	swelling	DMAP
□	original	–	–
△	original	+	–
○	original	+	+
■	reprecipitated	–	–
▲	reprecipitated	+	–
●	reprecipitated	+	+

Fig. 3 Acetylation of original and reprecipitated α-chitins. Chitin, 50 mg; pyridine, 10 mL; swelling, at room temperature for 24 h; DMAP (dimethylaminopyridine), 45 mg; acetic anhydride, 2.51 g; reaction temperature, rt

Fig. 4 Influence of swelling time on the acetylation of reprecipitated α-chitin (*solid circle*) and original α-chitin (*solid square*) at room temperature for 24 h



on the reaction was further examined in detail with samples swollen at room temperature for various periods. As indicated in Fig. 4, the reprecipitated chitin (solid circles) showed a sharp rise in the reactivity with increasing swelling time, although the increase of the ds value was the modest with the original chitin (solid squares). The enhanced reactivity of reprecipitated chitin is thus ascribable to the high swelling ability and surface area of the fluffy state as a result of reprecipitation under appropriate conditions.

Conclusions

Dissolution of chitin, especially α-chitin, in methanol/calcium chloride followed by dialysis and freeze-drying was confirmed to be a simple, convenient, and reliable procedure for the formation of a highly fluffy material from the dense solid of ordinary chitin. As expected, the resulting reprecipitated chitin exhibited much improved adsorption and reactivity. It has therefore proved a promising key starting material and should be intensely practical for controlled chemical modifications and various applications.

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