Polym. Bull. (2009) 62:119–126
DOI 10.1007/s00289-008-0002-2

ORIGINAL PAPER

Amorphous reprecipitated chitosan as a novel morphological form

Kazunori Okano · Takahiro Minagawa · Jin Yang · Manabu Shimojoh · Keisuke Kurita

Received: 19 August 2008 / Revised: 28 October 2008 / Accepted: 28 October 2008 /

Abstract In order to overcome the intractable nature of chitosan, which has delayed the basic and utilization research of this biopolymer having remarkable biological activities, destruction of the crystalline structure was studied. Chitosan was dissolved in aqueous acid, reprecipitated in alkali, and freeze-dried under appropriate conditions to prepare a fluffy cotton-like material, which was amorphous and had almost the same molecular weight as the original chitosan. It exhibited highly improved chemical reactivity as confirmed by the acetylation and enhanced adsorption capability toward copper ion. Full *N*-acetylation of the reprecipitated chitosan afforded structurally uniform chitin, which was also found to be amorphous. These results indicate the high potential of this new morphological form of chitosan possibly leading to a wide range of utilizations of this under-utilized biological resource.

Keywords Chitosan · Reprecipitation · Crystallinity · Amorphous chitosan · Amorphous chitin · Chemical modification · Advanced utilization · Metal adsorption

---

K. Okano · T. Minagawa · J. Yang · K. Kurita (✉)
Department of Materials and Life Science,
Faculty of Science and Technology, Seikei University,
Musashino, Tokyo 180-8633, Japan
e-mail: kurita@st.seikei.ac.jp

M. Shimojoh
Research and Development Department,
Toyo Suisan Kaisha, Ltd, Kohnan, Minato-ku,
Tokyo 108-8501, Japan

Introduction

Chitin is almost as abundant as cellulose and regarded as one of the most promising biopolymers because of its distinctive properties as an amino polysaccharide in addition to the environmentally benign nature [1–3]. The derived chitosan is considered to have even higher potential in view of the significant biological functions owing to the free amino groups. As the unique properties of chitosan are being disclosed, increasingly much attention has been paid to its advanced utilization in various fields, particularly in medicine, cosmetics, food processing, and agriculture [4, 5]. Chitosan is, however, soluble only in aqueous acid, and moreover, it is isolated as a dense crystalline solid. These characteristics are responsible for the difficulty in fabrication and chemical modifications and have undoubtedly restricted utilization, despite its high potentials [6–8].

It is therefore necessary to overcome its intractable nature for promoting both basic and utilization studies. If the crystalline structure could be destroyed effectively without any serious degradation, chitosan would become more accessible and useful. Crystallinity of chitosan was reduced to 10% on reprecipitation from a citric acid solution by adjusting the pH to 4.5 with sodium hydroxide [9]. To interfere with the strong intermolecular forces thoroughly, we examined reprecipitation under various conditions and evaluated the resulting reprecipitated chitosan. This paper reports a successful method to produce highly fluffy cotton-like chitosan, which proved to be much superior to the original chitosan as a functional polymer in many respects.

Experimental

General

IR spectra were recorded on a Shimadzu FTIR-8900 instrument by the KBr method. 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. Solvents were purified in usual manners and stored over molecular sieves. All the chemicals were of reagent grade and used without further purification.

Chitosan

Shrimp chitin was deacetylated with 40% aqueous sodium hydroxide at 110 °C for 4 h in nitrogen. After pulverization of the resultant chitosan, the alkaline treatment was repeated two more times to achieve complete deacetylation with a degree of deacetylation (dd) of 1.00 as confirmed by conductometric titration.

Molecular weight measurements

Chitosan was dissolved in 3% aqueous acetic acid and subjected to GPC measurement with a Shimadzu LC-10AD (column, Shodex OHpak SB-G + Shodex OHpak SB-804 HQ; solvent, CH_3CO_2H (0.07 mol/L)/CH_3CO_2Li (0.05 mol/L)/H_2O; flow rate, 0.5 mL/min) at 40 °C. The molecular weights were calibrated with pullulan standards.

Reprecipitation of chitosan

Chitosan (2.00 g) was dissolved in 200 mL of 2% aqueous acetic acid, and the solution was added to 400 mL of 5% aqueous sodium hydroxide dropwise slowly with vigorous stirring. The resulting swollen precipitate was collected on a paper filter at atmospheric pressure and washed repeatedly with deionized water until neutral. The precipitate was dispersed in 300 mL of deionized water and freeze-dried. The yield of white fluffy chitosan was 1.84 g.

Acetylation

To 50 mg (0.31 mmol) of the above-obtained reprecipitated chitosan were added 10 mL of pyridine and 3.16 g of acetic anhydride (31 mmol). The mixture was stirred at room temperature for 48 h in a nitrogen atmosphere and poured into ice water. The fibrous precipitate was collected by centrifugation, washed with deionized water and then with acetone, and dried to give 40 mg of the product. The degree of substitution (ds) for the acetyl group was 1.76 as calculated from the C/N ratio of elemental analysis. IR (KBr): ν 1736 (ester C=O), 1657 (amide I), 1556 (amide II), and 1150–1000 cm^{-1} (pyranose). Anal. Calcd for $(C_{10}H_{15}NO_6)_{0.76}(C_8H_{13}NO_5)_{0.24}\cdot 0.7H_2O$: C, 46.15; H, 6.48; N, 5.65. Found: C, 46.20; H, 6.31; N, 5.66.

N-Acetylation

In 100 mL of methanol, 500 mg of reprecipitated chitosan and 50 mL of acetic anhydride were added, and the mixture was kept at 50 °C for 48 h in nitrogen with stirring. The product was isolated in ice water, washed with deionized water and acetone, and dried. It was then treated with 1.4 g of potassium hydroxide dissolved in 250 mL of methanol at room temperature for 5 h, washed with methanol, and dried to give 0.46 g (75%) of selectively *N*-acetylated chitosan as a white fluffy material. IR (KBr): ν 1657 (amide I), 1558 (amide II), and 1150–1000 cm^{-1} (pyranose). Anal. Calcd for $C_8H_{13}NO_5\cdot 0.3H_2O$: C, 46.06; H, 6.57; N, 6.71. Found: C, 46.18; H, 6.50; N, 6.65.

Adsorption of copper ion

To 25.0 mL of 0.5 mmol/L aqueous copper(II) chloride solution was added 10 mg of chitosan, and the mixture was shaken at room temperature for a prescribed time. It was filtered with a glass filter, and the copper content in the supernatant was determined by UV spectroscopy using diethyldithiocarbamic acid [10].

Fig. 1 Pictures of original (*left*) and reprecipitated (*right*) chitosans of the same weight

Results and discussion

Reprecipitation of chitosan

Thoroughly deacetylated chitosan (dd 1.00) was used to avoid structural uncertainty, and the solution in 2% aqueous acetic acid was added to aqueous sodium hydroxide. To precipitate chitosan as a fluffy material, controls of the concentrations of chitosan and alkali, the reprecipitation mode, and the filtration method were found to be critical.

When the concentrations were too high or a considerable amount of chitosan solution was poured at a time, chitosan precipitated out as a heavy gel. However, when a chitosan solution of a proper concentration (about 1% chitosan in 2% aqueous acetic acid) was added dropwise slowly into 5% sodium hydroxide with agitation, a fibrous precipitate could be formed. On filtration under reduced pressure, however, a massive solid resulted, and the precipitate should thus be filtered on a filter paper at atmospheric pressure. After washing with water, the precipitate was suspended in water and freeze-dried to give chitosan as a white fluffy cotton-like material. If the amount of water for freeze-drying was not appropriate, only a dense solid or a fine powder was obtained. Figure 1 shows a picture of the reprecipitated chitosan as compared to that of the original chitosan of the same weight. The IR spectrum and elemental analysis supported the structure of chitosan.

Crystallinity

X-ray diffractometry by the powder method of the resulting chitosan was carried out to examine the influence of reprecipitation on the crystallinity. As evidenced in Fig. 2, no peaks due to the crystalline structure were observed for the reprecipitated chitosan. This indicates the effective destruction of the crystalline structure caused by the intrinsic strong intermolecular forces, resulting in the formation of amorphous chitosan.

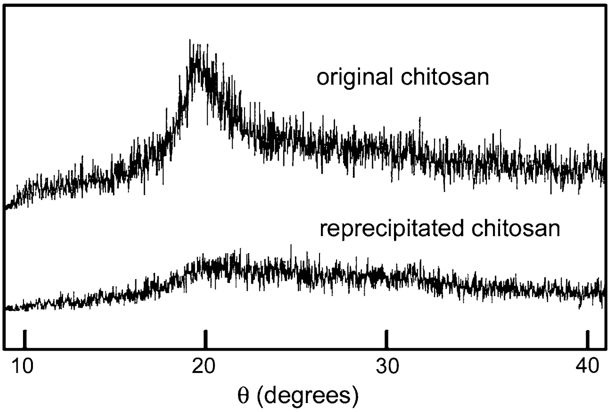


Fig. 2 X-ray diffraction diagrams of original and reprecipitated chitosans

Table 1 Molecular weights of original and reprecipitated chitosans by GPC[a]

	*M*n	*M*w	*M*w/*M*n
Original chitosan	40,100	218,000	5.44
Reprecipitated chitosan	41,200	213,000	5.17

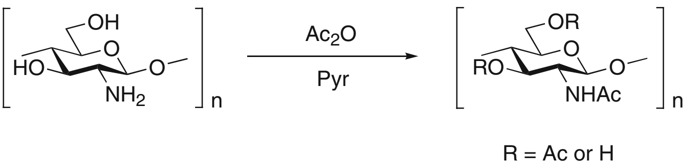
[a] Pullulan standards

Molecular weight characteristics

It was then considered essential to elucidate the molecular weight change during dissolution-reprecipitation. The molecular weight characteristics as determined by GPC of the reprecipitated chitosan were similar to those of the original chitosan as listed in Table 1, implying that the chitosan main chain suffered no appreciable extent of degradation in this process. This is most likely ascribable to the mild conditions, though a small reduction in the molecular weight was observed in the trimethylsilylation–detrimethylsilylation of chitin [11].

Acetylation reaction

Because of the fluffy appearance and amorphous nature, the reprecipitated chitosan would exhibit enhanced chemical reactivity. As a typical reaction of chitosan, acetylation was examined under various conditions to evaluate the reactivity (Scheme 1). As expected, the reprecipitated chitosan showed high reactivity compared to the original chitosan as summarized in Fig. 3. The addition of 4-dimethylaminopyridine (DMAP) was effective for both original and reprecipitated chitosans. The solid symbols representing the reactions of reprecipitated chitosan in the figure confirm a marked improvement of the reactivity, and acetylated derivatives with a ds around 2.9 were easily prepared even under these mild reaction conditions. Although swelling treatment in pyridine prior to the reaction was effective to some extent, the influence was not pronounced. In methanol as a solvent, the ds value was around 1 and increased only slightly with an increase in reaction time, owing most probably to the preferential reaction at the amino groups in this solvent (Fig. 3).



Scheme 1 Acetylation of chitosan

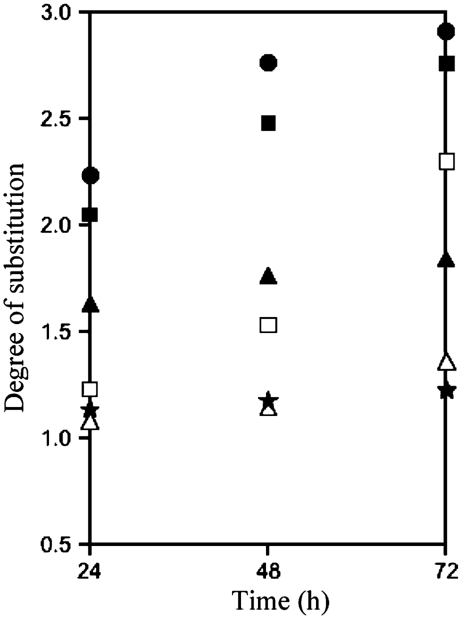


Fig. 3 Acetylation of original and reprecipitated chitosans with acetic anhydride under the reaction conditions shown below

	chitosan	solvent	swelling[a]	DMAP[b]
△	original	pyridine	–	–
□	original	pyridine	–	+
▲	reprecipitated	pyridine	–	–
■	reprecipitated	pyridine	–	+
●	reprecipitated	pyridine	+	+
★	reprecipitated	methanol	–	–

[a] At room temperature for 24 h prior to the reaction.

[b] 4-Dimethylaminopyridine.

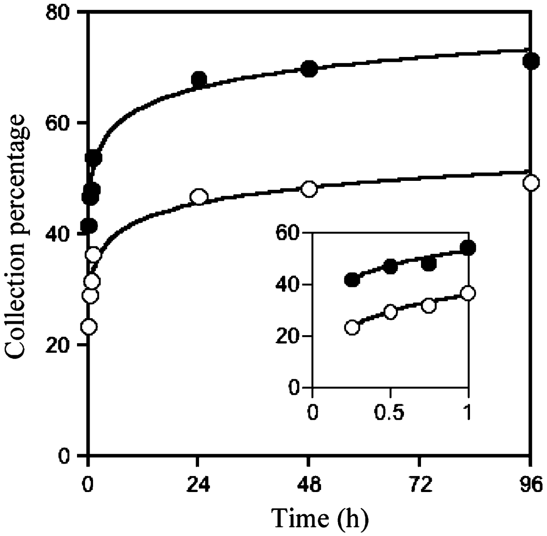


Fig. 4 Adsorption of copper (II) by original (*open circle*) and reprecipitated (*filled circle*) chitosans

Preparation of fully *N*-acetylated chitosan or fully *N*-acetylated chitin

Poly(*N*-acetyl-D-glucosamine), fully *N*-acetylated chitin, is important as a structurally pure chitin to discuss the structure-property relationship as well as to prepare chitin derivatives with well-defined structures. However, native chitin is partially *N*-deacetylated to varying extents, and ordinary chitin has a dd value of 0.1–0.2 due to some additional deacetylation during the isolation procedure. Though we have reported the synthesis of fully *N*-acetylated chitin, the starting materials were 50% deacetylated chitosan [12] or *β*-chitin [13], which are less common resources.

The high reactivity of reprecipitated chitosan suggested the possibility of efficient acetylation in methanol suitable for *N*-acetylation, and the reaction actually proceeded smoothly resulting in the formation of acetylated products with a ds value a little over 1.0 at room temperature to 50 °C. A small amount of *O*-acetyl groups could subsequently be removed selectively by transesterification to give the product as a fluffy material. The IR spectrum was identical with that of the authentic fully *N*-acetylated chitin. The ds was confirmed to be 1.0 by elemental analysis, and the conductometric titration indicated complete absence of free amino groups. These results supported that the product was regioselectively *N*-acetylated chitosan or structurally pure chitin. The product obtained here also showed no crystalline peaks in the X-ray diffraction diagram and is interesting as an amorphous form of chitin.

Adsorption of copper ion

The amorphous nature of reprecipitated chitosan should be associated with the adsorption behavior toward heavy metals. The adsorption of copper(II) ion by reprecipitated chitosan was thus measured, and the results are summarized in Fig. 4. As shown there, the reprecipitated chitosan was much superior to the original chitosan as an adsorbent in both the adsorption rate and capacity.

Conclusions

Reprecipitation of chitosan under appropriate conditions gave rise to a highly fluffy cotton-like material without a reduction in molecular weight. The product was amorphous in contrast to the original chitosan and exhibited much improved reactivity, indicating the product to be a convenient starting material for a wide variety of modification reactions even under heterogeneous reaction conditions. Fully *N*-acetylated chitin could be derived from this amorphous chitosan and is considered important as completely amorphous chitin. The reprecipitated chitosan is also expected to be much more useful as an adsorbent than the ordinary chitosan. Consequently, the reprecipitated chitosan prepared in this study proved to be a worthwhile new form of chitosan with high potentials in various fields.

Acknowledgments This work was partially supported by “High-Tech Research Center” Project for Private Universities: matching fund subsidy from MEXT, 2004–2008.

References

1. Roberts GAF (1992) Chitin chemistry. Macmillan, London
2. Kurita K (1997) Chitin and chitosan derivatives. In: Arshady R (ed) Desk reference of functional polymers: syntheses and applications. American Chemical Society, Washington DC, pp 239–259
3. Uragami T, Tokura S (eds) (2006) Material science of chitin and chitosan. Kodansha Scientific, Tokyo
4. Uragami T, Kurita K, Fukamizo T (eds) (2001) Chitin and chitosan in life science. Kodansha Scientific, Tokyo
5. Domard A, Guibal E, Vårum KM (eds) (2007) Advances in chitin science, vol 9. 10th ICCC-EUCHIS’06, Montpellier
6. Kurita K (2001) Prog Polym Sci 26:1921
7. Kurita K (2006) Mar Biotechnol 8:203
8. Kurita K (2006) Introduction of biologically active branches through controlled modification reactions of chitin and chitosan. In: Uragami T, Tokura S (eds) Material science of chitin and chitosan. Kodansha Scientific, Tokyo
9. Trung TS, Ng CH, Stevens WF (2003) Biotechnol Lett 25:1185
10. Kurita K, Koyama Y, Taniguchi A (1986) J Appl Polym Sci 31:1169
11. Sugita K, Yang J, Shimojoh M, Kurita K (2008) Polym Bull 60:449
12. Kurita K, Sannan T, Iwakura Y (1979) Makromol Chem 178:2595
13. Kurita K, Ishii S, Tomita K, Nishimura S, Shimoda K (1994) J Polym Sci Part A Polym Chem 32:1027