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Reactivity characteristics of a new form of chitosan

Facile *N*-phthaloylation of chitosan prepared from squid β-chitin for effective solubilization

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SUMMARY

A new form of chitosan prepared by deacetylating squid β-chitin was subjected to *N*-phthaloylation to evaluate the reactivity as compared with that of the conventional chitosan prepared from shrimp α-chitin. The reaction proceeded much more efficiently with squid chitosan than shrimp chitosan to give *N*-phthaloylchitosan, indicating considerable differences in higher-order structures between the two kinds of chitosans. Squid chitosan thus proved to show enhanced reactivity and be superior to shrimp chitosan as a starting material for controlled regioselective chemical modifications of chitin.

INTRODUCTION

Chitin is the second most abundant organic biomass resource next to cellulose. It is a quite attractive biopolymer having high possibilities of developing novel types of materials with advanced functions including specific biological activities, biocompatibility, biodegradability, and so on. Studies on chitin chemistry are thus expanding quite rapidly recently, but most of them have been based on α-chitin isolated from crab or shrimp shells owing to the easy accessibility.

There is another form of chitin, β-chitin. It has, however, not been prepared in quantity, and little is known on the chemistry. Squid pens are probably the most easily accessible source for β-chitin and will become increasingly important in view of supplying the enough amount of chitin with different higher-order structure to enable full exploration. In contrast to the strong intermolecular hydrogen bonding of α-chitin, responsible for intractability and poor reactivity, relatively weak intermolecular forces of β-chitin lead to enhanced swelling and solubility (1-3). It actually shows higher reactivity in deacetylation reaction, and under appropriate mild conditions, chitosan of high quality can be prepared from β-chitin (4). The resulting chitosan from β-chitin is considered of interest not only as an

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alternative chitosan source but also as a novel chitosan form having characteristics possibly different from those of the conventional chitosan prepared from α-chitin.

We have thus examined the reactivity of squid chitosan in phthaloylation to give *N*-phthaloylchitosan, that has proved to be quite useful as a soluble precursor for regioselective chemical modifications (5), and compared it with that of shrimp chitosan to elucidate the potential of squid chitosan as a starting material suitable for full exploration of chitin and chitosan.

EXPERIMENTAL

General

β-Chitin was isolated from pens of *Ommastrephes bartrami* by treating with 1 mol/L hydrochloric acid and with 2 mol/L sodium hydroxide as reported previously (4). Shrimp α-chitin was isolated from the shells of *Penaeus japonicus* (6). The chitin samples were pulverized with an ultra-centrifugal mill Retsch ZM-1 to give white materials. The degrees of deacetylation were 0.08 for β-chitin and 0.12 for α-chitin. IR spectra were taken on a JASCO IR-700 or JEOL JIR-3510. NMR spectra were recorded with a JEOL JNM-GX-270. Elemental analysis was performed with a Yanaco MT-3 CHNcorder. The degree of deacetylation was determined by conductometric titration with a TOA CM-40S.

Chitosans

Squid β-chitin was treated with 40% aqueous sodium hydroxide at 80°C for 3 h under nitrogen. The alkaline treatment was repeated two more times to give squid chitosan with a degree of deacetylation of 0.97-0.99 (4). Shrimp chitosan was prepared by repeating the alkaline treatment of α-chitin with 40% sodium hydroxide at 130°C under nitrogen two times. The degree of deacetylation was 0.99-1.0.

Phthaloylation

To a suspension of 0.500 g of squid chitosan in 20 mL of *N,N*-dimethylformamide (DMF) was added 1.38 g (3 molar equivalents to the amino groups) of phthalic anhydride, and the mixture was heated at 130°C under nitrogen with stirring. The solid went into solution in 0.5 h to give a light reddish brown clear solution, and the heating was discontinued after 2 h. The solution was cooled to room temperature and poured into ice-water. The resulting precipitate was filtered, washed with water, and dried. It was extracted with ethanol in a Soxhlet extractor for 8 h and dried to give 0.778 g of *N*-phthaloylchitosan as a light tan solid. IR(KBr): 3474 (O-H), 1776, 1710, 1393, and 719 (phthalimide C=O), and 1150-1000 cm^{-1} (pyranose). *Anal.* Calcd for $(C_{14}H_{13}NO_6)\cdot 0.6H_2O$: C, 55.67; H, 4.74; N, 4.64. Found: C, 55.87; H, 4.82; N, 4.36.

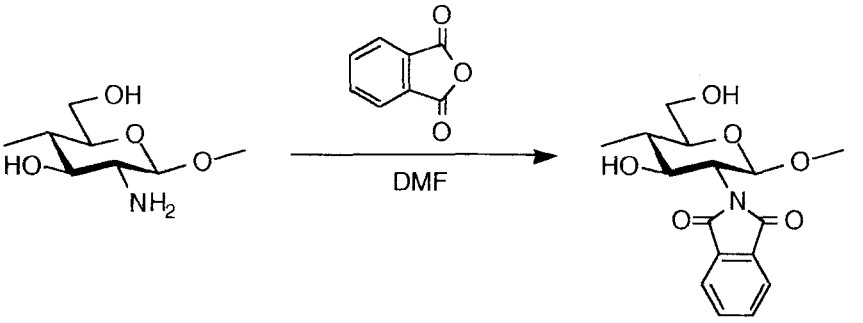
When the reaction was carried out at 130°C for 5 h, 0.975 g of the product was obtained. The substitution degree of phthaloyl groups per pyranose unit was calculated to be 1.4 from the elemental analysis data. *Anal.* Calcd. for $(C_{14}H_{13}NO_6)_{0.6}(C_{22}H_{17}NO_9)_{0.4}\cdot 1.2H_2O$: C, 55.52; H, 4.60; N, 3.76. Found: C, 55.20; H, 4.31; N, 3.92.

Acetylation of the phthaloyl derivative

Phthaloylated chitosan prepared by 5 h reaction at 130°C described above, 0.500 g, was dissolved in 20 mL of pyridine, and 10 mL of acetic anhydride was added. The solution was stirred at room temperature for 12 h and then poured into ice-water. The precipitated solid was collected by filtration, washed with water and then ethanol, and dried to give 0.580 g of the peracetylated product as a pale tan solid. IR (KBr): 1775, 1717, 1388, and 721 (phthalimide C=O), 1745 (ester C=O), and 1150-1000 cm^{-1} (pyranose). *Anal.* Calcd for $(C_{18}H_{17}NO_8)_{0.6}(C_{24}H_{19}NO_{10})_{0.4}\cdot 0.8H_2O$: C, 56.70; H, 4.52; N, 3.24. Found: C, 56.98; H, 4.74; N, 3.25.

RESULTS AND DISCUSSION

Both squid and shrimp chitosans were obtained as almost colorless powdery materials. *N*-Phthaloylation reaction of squid chitosan was carried out under various conditions to evaluate the reactivity and compared with that of shrimp chitosan.



When squid chitosan, suspended in DMF, was treated with excess phthalic anhydride at 100°C, it went into solution in 6-10 h to give a clear viscous solution. At 130°C, dissolution was complete in 0.5 h. The products isolated after 24 h at 100°C or 0.5 h at 130°C were light yellow to pale tan solids. The IR spectra of the products, however, showed incomplete cyclization of *N*-phthaloyl groups as evident by the presence of weak absorption bands at around 1650 and 1560 cm^{-1} attributable to amide linkages, besides the characteristic bands due to phthalimide groups. Moreover, the yield obtained after 0.5 h reaction at 130°C was low as shown

in Table 1. Heating for 2 or 3 h at 130°C resulted in complete *N*-phthaloylation as supported by IR spectroscopy and elemental analysis. The resulting *N*-phthaloylchitosan was obtained as a light tan powdery material and readily soluble in common polar organic solvents including DMF, dimethyl sulfoxide, and pyridine.

On prolonged phthaloylation over 5 h at 130°C, however, the product showed weak broad bands in the IR spectrum at around 2500 cm^{-1} due to carboxyl groups. Furthermore, the yield was more than 100% based on the formation of *N*-phthaloylchitosan. These results suggest that phthaloylation occurred to some extent at the hydroxyl groups, probably those at the C-6 positions, in addition to the amino groups. The degree of substitution estimated from elemental analysis was 1.4. In order to confirm the structure of the product, it was fully acetylated with acetic anhydride in pyridine solution. The elemental analysis data of the peracetyl derivative again confirmed the degree of phthaloylation to be 1.4. The peracetyl derivative showed remarkable solubility and was soluble even in low boiling solvents such as dichloromethane and chloroform. The degree of phthaloylation was thus able to be calculated by ^{1}H-NMR spectroscopy in chloroform-*d*. The area ratio of peaks due to acetyl groups at 1.70 and 1.95 ppm to peaks due to phthalimide groups at 7.6-7.8 ppm was 1:1.20, corresponding to a degree of phthaloylation of 1.4.

Table 1
N-Phthaloylation of Squid and Shrimp Chitosans in DMF[a]

Source	Temp, °C	Time, h	Appearance of mixture	Yield, %
Squid	100	24	homogeneous	85
	130	0.5	homogeneous	49
	130	1	homogeneous	88
	130	2	homogeneous	86
	130	3	homogeneous	91
	130	5	homogeneous	108
Shrimp	100	48	heterogeneous	5
	130	7	homogeneous	96

[a] Chitosan, 0.500 g; phthalic anhydride, 3 molar equiv; DMF, 20 mL.

When the conventional chitosan prepared from shrimp α-chitin was heated with phthalic anhydride in DMF at 130°C in a similar manner, it took 4-5 h for dissolution of chitosan and 5-7 h for complete *N*-phthaloylation as reported previously (5). Phthaloylation of shrimp chitosan was also attempted at 100°C as in the case of squid chitosan. Most of the chitosan,

however, did not go into solution even after 48 h as shown in Table 1 and was recovered as such. These results clearly indicate the marked difference in reactivity between the two chitosans.

CONCLUSION

A new form of chitosan prepared from squid β-chitin proved to show much higher reactivity in phthaloylation than the conventional chitosan from shrimp α-chitin, and *N*-phthaloylchitosan could be prepared in a more facile manner. The high reactivity of squid chitosan is probably associated with loose arrangement of the chitosan molecules as suggested by the amorphous x-ray diffraction pattern (4) in sharp contrast to the crystalline nature of shrimp chitosan (6). It is worth noting that the difference in the higher-order structures between the two kinds of chitins, α- and β-chitins, remains considerably even after conversion to chitosans by alkaline deacetylation. These results imply high potentials of squid chitosan as a starting material capable of facile and regioselective modification reactions to afford derivatives with well-defined structures.

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