Communications

Chemoselective Protection of the Amino Groups of Chitosan by Controlled Phthaloylation: Facile Preparation of a Precursor Useful for Chemical Modifications

Keisuke Kurita,*† Hiroyuki Ikeda,† Yuya Yoshida,† Manabu Shimojoh,‡ and Manabu Harata†

Department of Applied Chemistry, Faculty of Engineering, Seikei University, Musashino-shi, Tokyo 180-8633, Japan, and Research and Development Department, Toyo Suisan Kaisha, Ltd., Kohnan, Minato-ku, Tokyo 108-8501, Japan

Received July 20, 2001; Revised Manuscript Received September 26, 2001

A simple and convenient procedure for chemoselectively protecting the amino groups of chitosan has been developed to provide N-phthaloyl-chitosan that is indispensable as a soluble N-protected precursor for further controlled modification reactions of chitosan. Although the conventional N-phthaloylation of chitosan in N,N-dimethylformamide was accompanied by partial phthaloylation of the hydroxy groups, the addition of a small amount of hydroxy-containing compounds effectively suppressed the O-phthaloylation. Of some compounds examined, water proved particularly suitable, resulting in the formation of chemoselectively N-phthaloylated chitosan without any appreciable O-phthaloyl groups. The resulting N-phthaloyl-chitosan was found to be crystalline despite the presence of a bulky substituent. A solubility test indicated that N-phthaloyl-chitosan exhibited considerable affinity for organic solvents.

Introduction

Despite unique biological activities and physicochemical properties,1 chitin still remains an unutilized biomass resource due to its intractable nature; it is insoluble in common solvents. In view of developing materials with advanced functions, many attempts have been made to modify the molecular structure of chitin and thereby to improve or control the properties.2 The reactions, however, often encounter difficulty because of the heterogeneous reaction conditions, multifunctionality with three kinds of functional groups, and poor reactivity. The products are therefore structurally ambiguous in many cases, and it is difficult to discuss the structure–property relationship.

Some soluble derivatives of chitin have proved useful for performing modification reactions in a facile and controlled manner.3 They include partially deacetylated water-soluble chitin,4 tosyl-chitin,5 iodo-chitin,5 and trimethylsilyl-chitin.6 Phthaloylated chitosan is a particularly important and indispensable organosoluble precursor.7 Treatment of chitosan with phthalic anhydride, however, generally results in partial O-phthaloylation in addition to the N-substitution.8 With the phthaloylated product as a key intermediate, various modification reactions proceed smoothly in solution,9–11 but the O-phthaloyl group is an obstacle in most cases to quantitative and regioselective substitution. Recently we reported on the removal of O-phthaloyl groups by transesterification,12 but it is desirable to avoid the use of alkali that might be harmful to the N-phthaloyl group. If N-phthaloyl-chitosan can be prepared in a one-step reaction, it would be highly beneficial for conducting a wide variety of modification reactions site-specifically and quantitatively to construct well-defined molecular architectures on chitosan. We report here a simple and reliable method to provide chemoselectively protected N-phthaloyl-chitosan and discuss some properties of the product.
Phthaloylation of Chitosan.

Protection of the functional groups of chitosan is crucial for conducting modification reactions in a well-controlled manner, and introduction of the phthaloyl group at the amine functionality is ideal not only for the protection but also for improving solubility. Furthermore, dephthaloylation is facile with hydrazine at 80 °C. Although the phthaloylation is accompanied by partial O-phthaloylation, the product is still a convenient organosoluble precursor for some modification reactions including acylation to prepare derivatives having liquid crystalline properties. However, to expand the scope of chemoselective and quantitative modification reactions of chitosan, it would be highly advantageous to use structurally well-defined N-phthaloyl-chitosan.

Phthaloylation Reaction. Phthaloylation of chitosan is usually performed in DMF with excess phthalic anhydride at 120–130 °C for 8 h. As shown in Table 1, the ds of the product obtained after 5 h reaction was 1.54. Interestingly, however, the ds decreased to some extent on prolonged reaction. This implies the possibility of hydrolytic cleavage of the once formed ester linkages with the water produced by N-phthaloylation.

The reaction was thus examined in a mixed solvent composed of DMF and a hydroxy-containing substance for the possibility of chemo-specific protection, and the results are included in Table 1. The ds values of the products prepared in DMF/ethanol and DMF/ethylene glycol were low. Although the product prepared in DMF showed weak bands at 1290 and 1260 cm⁻¹ (ester) in the IR spectrum in Figure 1 (free carboxyl) and medium ones at 1,712 (imidic C=O) and 1,297 cm⁻¹ (imidic N=O), the mixture was heated in nitrogen at 120 °C with stirring. After 8 h of reaction, the resulting pale tan mixture was cooled to room temperature and poured into ice water. The precipitate was collected on a filter, washed with 150 mL of methanol at room temperature for 1 h, and dried to give 0.444 g of the product as a pale tan powder. The degree of substitution (ds) was determined to be 1.00 from the C/N value of elemental analysis (see Table 1), and the possibility of chemospecific protection, and the results are included in Table 1. The ds values of the products prepared in DMF/ethanol and DMF/ethylene glycol were low. Although the product prepared in DMF showed weak bands at 2600–2700 cm⁻¹ (free carboxyl) and medium ones at 1290 and 1260 cm⁻¹ (ester) in the IR spectrum in Figure 1, the products obtained in these mixed solvents showed weak bands at 1290 and 1260 cm⁻¹. This suggests incomplete cyclization at the amino group and/or partial O-phthaloylation though to only a small extent. Methyl Cellosolve appeared to be better as a cosolvent, judging from the IR spectrum of the product, but the ds was still less than 1.0.

Of the four cosolvents examined, water proved the most appropriate; the product was much lighter in color, and}

<table>
<thead>
<tr>
<th>Table 1. Phthaloylation of Chitosan in Various Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>solvent (v/v)</td>
</tr>
<tr>
<td>DMF</td>
</tr>
<tr>
<td>DMF</td>
</tr>
<tr>
<td>DMF</td>
</tr>
<tr>
<td>DMF/ethanol (95/5)</td>
</tr>
<tr>
<td>DMF/ethylene glycol (95/5)</td>
</tr>
<tr>
<td>DMF/Methyl Cellosolve (95/5)</td>
</tr>
<tr>
<td>DMF/water (95/5)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The reaction was carried out with 0.300 g of chitosan and 3 equiv of phthalic anhydride in 6 mL of solvent at 120 °C. <sup>b</sup> Degree of substitution calculated from the C/N value of elemental analysis.

**Experimental Section**

**General Information.** IR spectra were recorded on a Shimadzu FTIR-8900 by the KBr method. <sup>13</sup>C cross polarization magic angle spinning (CP/MAS) NMR spectra were taken with a JEOL JNM-LA400D at 20 °C and <sup>13</sup>C frequency of 100.40 MHz with TOSS (total suppression of sidebands) and TOSDL (TOSS and dipolar-dephasing) modes using hexamethylbenzene (17.36 ppm) as the external standard. 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 instrument. Phthalic anhydride was purified in usual manners and stored over molecular sieves.

**Results and Discussion**

Phthaloylation of Chitosan.

Phthaloylation of chitosan is crucial for conducting modification reactions in a well-controlled manner, and introduction of the phthaloyl group at the amine functionality is ideal not only for the protection but also for...
furthermore, the ds of the product was confirmed to be 1.0 (Scheme 1). These data indicate that DMF/water is by far the most suitable for phthaloylation in terms of the ds value as well as the reaction selectivity, and the chemoselective full N-phthaloylation became possible in a simple manner. The yield was in a range 80–90%.

The IR spectrum (Figure 1C) of the product obtained in DMF/water shows bands assignable to N-phthaloyl-chitosan and no appreciable bands due to free carboxyl groups. The twin absorptions at 1776 and 1712 cm$^{-1}$ are characteristic of imide; it should be noted that the latter is particularly sharp in comparison with that in spectrum A, supporting the absence of the ester carbonyl.

The solid-state $^{13}$C NMR spectra of the product are shown in Figure 2. The spectrum in the TOSS mode exhibits peaks assignable to N-phthaloyl-chitosan. In the TOSDL mode, peaks due to CH and CH$_2$ should disappear because of the short relaxation times, and as expected, only two peaks ascribable to the carbonyl and C-1,2 of phthaloyl could be observed. Moreover, the peaks in the spectra of both modes were rather sharp and well resolved compared to those of the products having additional O-phthaloyl groups.

**Some Properties.** As shown in Figure 3, fully deacetylated chitosan prepared by repeated alkali treatments showed lower crystallinity than the ordinary chitosan of around 95% deacetylation.$^{14}$ The phthaloylated product prepared in DMF was amorphous, because of the heterogeneous structure owing to partial O-substitution in addition to the bulkiness of the phthaloyl group. In sharp contrast, N-phthaloyl-chitosan prepared in DMF/water showed certain crystallinity as evidenced by diagram C in Figure 3, despite the introduction of the phthaloyl group; this also supports the uniform structure of the product. It is noteworthy that N-phthaloyl-chitosan exhibited crystallinity even though such a bulky substituent had been introduced.

A qualitative solubility test indicated that the resulting N-phthaloyl-chitosan was soluble in some organic solvents

![Scheme 1](image)

![Figure 2](image)

![Figure 3](image)
including m-cresol, dichloroacetic acid, N,N-dimethylacetamide/8% LiCl,\textsuperscript{15} and methanol/CaCl\textsubscript{2}.\textsuperscript{16} It swelled in more common solvents such as pyridine, DMF, and dimethyl sulfoxide that dissolved the product having additional O-phthaloyl groups. The relatively low solubility of N-phthaloyl-chitosan may be partly attributable to some crystallinity as suggested by X-ray diffractionmetry.

Conclusions

Chemoselective N-phthaloylation of chitosan could be accomplished successfully in one step using DMF containing 5% water as a solvent. It is rather surprising that crystallinity was observed with N-phthaloyl-chitosan. Crystallization of chitin or chitosan derivatives, particularly those having bulky substituents, is usually difficult, and N-phthaloyl-chitosan appears to be the first example of a derivative with a bulky substituent that can crystallize. The N-phthaloyl-chitosan exhibited high affinity for organic solvents, although somewhat lower than that of the product having additional O-phthaloyl groups. The simple procedure established here enables facile preparation of N-phthaloyl-chitosan, a convenient precursor for the construction of sophisticated molecular architectures based on the specialty biopolymer chitosan.

Acknowledgment. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 12650872) from the Ministry of Education, Science, and Culture of Japan and by a grant from Towa Shokuhin Kenkyu Shinkoukai.

References and Notes