Preparation and Evaluation of Trimethylsilylated Chitin as a Versatile Precursor for Facile Chemical Modifications

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Trimethylsilylation of chitin was studied in detail to establish a reliable method, and the properties of the resulting product were elucidated. Chitin was successfully trimethylsilylated with a mixture of hexamethyldisilazane and trimethylsilyl chloride in pyridine. Compared to \( \alpha \)-chitin, \( \beta \)-chitin was much more reactive and advantageous as a starting material to prepare fully substituted chitin in a simple manner, though \( \alpha \)-chitin also underwent full silylation under appropriate conditions. The resulting silylated chitin was characterized by marked solubility in common organic solvents and by easy desilylation to regenerate hydroxy groups, which enabled clean preparation of chitin films. The reactivity of the silylated chitin was examined by treating with triphenylmethyl chloride and acetic anhydride as typical alkylation and acylating reagents, and complete substitutions were readily accomplished. The silylated chitin has thus proved to be a superb precursor for modification reactions.

Introduction

Cellulose and chitin are the two most abundant organic compounds on earth, boasting huge annual productions. They are structurally similar to each other, but chitin has unique characteristics because of the C-2 acetamide groups.\(^{1-3}\) Increasingly more attention has been paid to chitin as its distinctive biological activities and physicochemical properties are being better understood. It is expected to have great potential as a specialty biopolymer useful in various fields, including medicine, cosmetics, food, and agrochemicals.

Despite the interesting properties of chitin, only a very limited amount is currently used, mostly in the deacetylated form, chitosan. Chemical modifications of chitin and chitosan diversify the molecular structures and hence properties, and recent progress in noteworthy.\(^{4}\) There are, however, serious obstacles to modification reactions due to the inherent properties of chitin such as limited solubility, poor reactivity, and multifunctionality.

To prepare derivatives with well-defined structures and thereby to develop advanced functional materials, it is crucial to manipulate chitin and chitosan in well-controlled manners. In this context, the water-soluble chitin\(^\text{5}\) and some organosoluble derivatives such as \( O \)-tosylchitin\(^\text{6}\) and \( N \)-phthaloyl-chitosan\(^\text{7}\) have proved useful for facile modification reactions under mild conditions in solution. Besides these soluble precursors, \( \beta \)-chitin is another candidate for efficient modifications, because of its relatively weak intermolecular forces\(^{8,9}\) compared to that of the ordinary \( \alpha \)-chitin.\(^{10}\) \( \beta \)-Chitin is thus expected to be superior as a starting material with higher processability, and in fact, it exhibits higher reactivity as confirmed in several reactions.\(^{11,12}\)

Chitin will become soluble provided the strong intermolecular hydrogen bonding could be interfered by proper substitution. Trimethylsilylation was considered to meet the requirement for solubilization in common organic solvents, and the silylated product would be a convenient precursor for fabrication as well as for structural modifications. It is therefore important to establish a procedure for full trimethylsilylation, and the possibility of direct silylation of \( \beta \)-chitin was shown in a preliminary communication.\(^{13}\) Here we report the results of detailed studies on the simple and reliable silylation method for \( \alpha \)- and \( \beta \)-chitins and on the evaluation of the silylated product as a versatile organosoluble precursor.

Experimental Section

General Procedures. IR spectra were recorded on a Shimadzu FTIR-8900 by the KBr method. \(^1\)H NMR spectra were taken with a JEOL JNM-LA400D in actone-\( d_6 \) at ambient temperature or in dimethyl sulfoxide-\( d_6 \) at 90 °C. Elemental analysis was performed with a Perkin-Elmer 2400. All the chemicals were of reagent grade and used without further purification. Solvents were purified in usual manners and stored over molecular sieves.

Chitins. \( \alpha \)-Chitin isolated from shrimp shells was purified as reported,\(^{14}\) and the degree of deacetylation (DD) was 0.10, as determined by conductometric titration with a TOA conductivity meter CM-40S. \( \beta \)-Chitin was isolated from squid pens,\(^{11}\) and the DD was 0.11. Both chitin samples were pulverized to 0.5 mm mesh with an ultracentrifugal mill (Retsch ZM-1). They were subsequently treated with acetic anhydride in methanol to acetylate the free amino groups by the method described previously.\(^{12}\) The DD values of the products were 0.0.

Trimethylsilylation of Chitin. \( \beta \)-Chitin (DD 0.0, 0.100 g, 0.493 mmol of pyranose) was added to 10 mL of pyridine,
and the dispersion was heated at 100 °C for 24 h. After cooling to room temperature, 1.03 mL (0.793 g, 4.93 mmol) of hexamethyldisilazane and 0.63 mL (0.535 g, 4.93 mmol) of trimethylsilyl chloride were added. The mixture was stirred at 70 °C for 48 h. The resulting pale brown solution was poured into 150 mL of acetone, and the mixture was centrifuged. The clear supernatant (acetone extract) was isolated and concentrated under reduced pressure. The resulting pale brown viscous oil was added dropwise to 500 mL of cold water to give a white fibrous precipitate. It was filtered, washed with water, and dried to yield 0.151 g (88%). The degree of substitution (DS) was 2.00 as confirmed by the C/N value of elemental analysis and 1H NMR spectroscopy. IR (KBr): ν 3328 (O–H), 2958 (C–H), 1664 (amide I), 1540 (amide II), 1150–1000 (pyranose), and 842 cm⁻¹ (Si–CH₃). 1H NMR (acetone-d₆): δ 0.00 (s, Si–CH₃, 18H), 1.84 (s, CO–CH₃, 3H), 3.20 (br s, pyranose, 1H), 3.65 (br m, pyranose, 5H), 4.28 (br s, pyranose, 1H), and 7.07 ppm (br s, N–H, 1H).

Anal. Calcd for C₁₄H₂₅NO₅Si₃: C, 48.38; H, 8.41; N, 4.03. Found: C, 48.06; H, 8.50; N, 3.97.

Film Casting. A solution of trimethylsilylated chitin in acetone was cast on a glass plate and allowed to dry at room temperature. The resulting film, about 1 × 10⁻² mm thick, was peeled, immersed in 10% aqueous acetic acid for 60 min, washed with water, and dried to give a transparent colorless film of chitin.

Triphenylmethylation of Silylated Chitin: Method 1. After evaporation of the acetone extract of the trimethysilylation mixture starting from 0.100 g (0.288 mmol of pyranose) of β-chitin as described above, the resulting oil was dissolved in 5 mL of pyridine. Triphenylmethyl (trityl) chloride (0.803 g, 2.88 mmol) was added, and the solution was stirred at 90 °C for 24 h in nitrogen. It was poured into 150 mL of methanol to precipitate a pale tan powder. The product was centrifuged. The clear supernatant (acetone extract) was isolated and concentrated under reduced pressure. The resulting pale brown viscous oil was added dropwise to 500 mL of cold water to give a white fibrous precipitate. It was filtered, washed with 2% aqueous acetic acid for 10 min and then with water, and dried to give an acetylated product as a pale tan powdery material. The DS for O-acetyl groups was 2.00, as determined from the C/N value of elemental analysis. The yield was 0.042 g (98%). IR (KBr): ν 3317 (N–H), 1746 (ester C=O), 1668 (amide I), 1543 (amide II), and 1150–1000 cm⁻¹ (pyranose).

Anal. Calcd for C₁₂H₁₇NO₇: C, 68.72; H, 6.03; N, 4.82. Found: C, 69.45; H, 6.22; N, 4.83.

Results and Discussion

Trimethylsilylation of Chitins. Trimethylsilylation of 3-O-acetyl-2-N-phthaloylchitosan was effective to markedly enhance the solubility in organic solvents, while considerable reactivity of the hydroxy functionality was still retained. Chitin was once reported to be trimethylsilylated with hexamethyldisilazane in formamide at 70 °C but only partially substituted (DS 0.6) under the conditions where cellulose was fully substituted (DS 3.0), indicating the relatively poor reactivity of chitin. Full trimethylsilylation of cellulose was also achieved in liquid ammonia. Our aim was to establish a simple procedure for preparing fully trimethylsilylated chitin, and the silylation reaction was thus examined with α- and β-chitins.

α-Chitin and β-chitin were isolated from shrimp shells and squid pens as a white powder and a cotton-like fluffy material, respectively, after ultracentrifugal pulverization. They had some free amino groups, which were selectively acetylated to give structurally uniform chitins.

The reaction was first attempted at room temperature with a mixture of hexamethyldisilazane and trimethylsilyle chloride in pyridine (Scheme 1) as in the trimethylsilylation of an N-phthaloylchitosan derivative. The substitution reaction proceeded under heterogeneous conditions in a dispersion state, and the product was isolated in methanol. As shown in Figure 1, the silylation was slow, and similar results were obtained from either α-chitin or β-chitin. The product obtained after 96 h reaction had a DS up to 0.5.

The reaction temperature was then raised as suggested by the trimethylsilylation of cellulose, amylose, and dextran. As expected, β-chitin underwent substitution smoothly at 70 °C, and the initially heterogeneous mixture became a homogeneous cloudy solution in about 5 h. The product was isolated after 24 h by pouring the solution into methanol as

![Scheme 1](image-url)
a swollen precipitate. The resulting solid had a DS of around 0.7 and was not soluble in pyridine anymore. This implied the possibility that the trimethylsilyl groups introduced were partly removed during the isolation process.

The isolation was thus examined in detail. Attempts to extract the silylated product from the reaction mixture were unsuccessful with solvents such as 1,4-dioxane, hexane, or diethyl ether and allowed only partial isolation with tetrahydrofuran or 1,2-dichloroethane. In contrast, acetone was found to extract the silylated product, effectively leaving the byproduct salts undissolved. The acetone extract was separated, concentrated, and poured into various solvents for precipitation. Of some solvents examined, 1,4-dioxane and water gave a product of high DS judging from the strong absorption bands due to Si-CH$_3$ at around 1250 and 840 cm$^{-1}$ in the IR spectra. However, 1,4-dioxane gave a swollen precipitate, and water proved more suitable, giving rise to a white, fibrous, fluffy precipitate. A combination of acetone and water was thus confirmed to be appropriate in view of the easy isolation of the highly silylated chitin as well as the thorough removal of the byproduct salts. The DS of the product isolated in this manner was high and reached 2.0 in 16 h at 70 °C with β-chitin, as shown in Figure 1.

The silylation was also conducted with α-chitin at 70 °C, but the reaction was much more sluggish than that with β-chitin, as expected from the results of other modification reactions.¹⁰,¹¹ As evident in Figure 1, though the DS was only 0.99 after 24 h, full substitution could be attained in 96 h, even with α-chitin.

Besides the satisfactory elemental analysis data, IR and NMR spectroscopies supported the structure of the silylated product. In the IR spectrum in Figure 2, characteristic bands are observed at 1251 and 840 cm$^{-1}$ ascribable to the Si-CH$_3$ bond. Figure 3 shows the $^1$H NMR spectrum of the silylated product. The peaks were referenced to the trimethylsilyl peak, and those due to acetyl methyl and amide N-H are found at 1.84 and 7.07 ppm, respectively. The pyranose C=H linkages are responsible for three broad peaks centering at 3.20, 3.65, and 4.28 ppm. The peak area at 0 ppm corresponded to 18 protons as compared to other peak areas.

**Properties of Trimethylsilylated Chitin.** Solubility of the resulting silylated chitin was dependent on the DS value. The highly substituted chitin showed particularly enhanced solubility in organic solvents. It was readily soluble in acetone and pyridine and partially soluble in the common polar solvents. When the DS was below 0.7, the product was not soluble but swelled as summarized in Table 1.

The marked solubility of the fully silylated product in organic solvents made film preparation quite easy; transparent films were obtained by the solution casting method from acetone. However, the Me$_3$Si-O bond is rather labile, and the stability was thus examined with the film in 10% aqueous acetic acid. As obvious in Figure 4, the desilylation reaction was extremely rapid even at room temperature in the film.
complete removal of the silyl groups. Tritylation proceeded even at 70 °C, but the reaction at 90 °C resulted in a higher DS, as listed in Table 2. The DS was up to 0.95 in 48 h reaction. DMAP appeared ineffective to improve the DS. With α-chitin, the DS of the product was low, suggesting the necessity of highly trimethylsilylated chitin for efficient substitution.

To accomplish complete discrimination of the functional groups in chemical modifications, full protection of the C-6 hydroxy groups is essential, and the tritylation was then examined with isolated and dried silylated chitin. The reaction was more efficient in this case, as expected, and the DS became 1.0 in 24 h as included in Table 2.

This preparative method for tritylated chitin via silylation is superior to direct tritylation or the procedure based on N-phthaloylchitosan. In attempts to directly tritylate either β-chitin or α-chitin, no appreciable extent of substitution was observed under the conditions where the DS 1.0 was achieved with silylated chitin, though DMAP was somewhat effective to improve the DS. Tritylated chitin could also be prepared from N-phthaloylchitosan as a key intermediate, but it takes a series of meticulous five-step reactions starting from chitin. These results indicate the usefulness of silylated chitin as an organosoluble precursor for modification reactions.

All the products showed satisfactory results of elemental analysis. The IR spectrum of the product in Figure 2 shows typical bands due to phenyl groups at 3062, 746, and 702 cm⁻¹ and no bands due to trimethylsilyl groups. The ¹H NMR spectrum of the tritylated chitin also supported the structure; multiplet peaks at 7.19—7.38 ppm are characteristic of trityl. Furthermore, the integration ratios of peaks agreed with the theoretical values. The yields were generally high and in the range of 80—90%. The tritylated chitin was soluble in polar organic solvents such as pyridine and dimethyl sulfoxide, in sharp contrast to the insoluble chitin (Table 1).

Acetylation of Silylated Chitin. Acylation is another important reaction for chitin to modify the chemical structure. However, even acetylation is not easy, because of the strong intermolecular forces. Though acetylation is possible under...
was a key step and found to be successful with acetone and water in view of the purity of the product, simplicity of isolation, and reproducibility. The resulting trimethylsilylated chitin is noteworthy in many respects: it is prepared in a straightforward way, it is readily soluble in common solvents, the trimethylsilylated hydroxy functionality exhibits considerable reactivity, and the trimethylsilyl group can be removed easily when necessary. All of these characteristics indicate the high potential of the silylated chitin in chemical modifications for constructing diversified molecular environments on the chitin backbone and also in the facile fabrication of this intractable polysaccharide.

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References and Notes


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Table 3. Acetylation of Chitins and Trimethylsilylated Chitin (TMS-chitin)\(^a\)

<table>
<thead>
<tr>
<th>chitin</th>
<th>Ac(_2)O (equiv)</th>
<th>additive(^b)</th>
<th>temp (°C)</th>
<th>time (h)</th>
<th>degree of O-Ac(^c)</th>
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<td>48</td>
<td>2.00</td>
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</table>

\(^a\) Trimethylsilylated chitin, DS 2.0, prepared from β-chitin. \(^b\) Six-fold mole of pyranose units; TEA, triethylamine; DMAP, 4-(dimethylamino)pyridine. \(^c\) Determined from the C/N value of elemental analysis.

Scheme 3

\[ \text{Me}_3\text{SiO} \text{OSiMe}_3\text{NaH} \rightarrow \text{Me}_3\text{SiO} \text{OSiMe}_3\text{AcO} \]

rather harsh conditions,\(^{21-23}\) it is accompanied by some undesirable side reactions. Direct acetylation of α-chitin with acetic anhydride in pyridine at room temperature was sluggish and gave a derivative with DS 0.22, as listed in Table 3. β-Chitin was more reactive, and the DS was 0.85 under the same conditions. As an additive, DMAP was more effective than triethylamine.

Acetylation was expected to be facile with silylated chitin and studied under various conditions (Scheme 3). The reaction proceeded in homogeneous solution in pyridine, and the product was isolated in water and washed with dilute acetic acid to ensure removal of silyl groups. The influence of the amount of acetic anhydride was examined first, and similar results were obtained with 25 and 50 equiv. With 50 equiv of acetic anhydride, the DS was confirmed to level off at 1.80–1.85 judging from the results of the reactions for 24, 48, and 72 h. On addition of DMAP, the reaction became facile; the DS was 1.28 in 2 h and reached 2.00 in 8 h. Full acetylation was also attained at 50 °C even in the absence of DMAP, as included in Table 3. The elemental analyses of the products were satisfactory. The acetylated products were obtained as off-white to pale tan powdery materials, and the yields were above 85% in general, despite the small-scale reactions.

Conclusions

Full trimethylsilylation was accomplished with either shrimp α-chitin or squid β-chitin, but the reaction was much more facile and easy with β-chitin. Isolation of the product