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Trimethylsilylation of chitosan and some properties of the product

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Abstract

Chitosan was trimethylsilylated, and some properties of the product such as solubility and reactivity were examined. Trimethylsilylation proceeded efficiently with a mixture of hexamethyldisilazane and chlorotrimethylsilane in pyridine; chitosan prepared from β-chitin underwent substitution more facilely than that from α-chitin. The degree of trimethylsilylation per pyranose unit reached 2.9. The resulting silylated derivative was almost soluble in pyridine and swelled considerably in common organic solvents. Though the product was hydrolytically stable in neutral and weakly alkaline media, it was deprotected readily with acid. As a typical example of modification reactions, acetylation of silylated chitosan was examined, and highly acetylated derivatives were obtained under mild conditions, indicating a high potential of the silylated chitosan as a precursor for chemical modifications of chitosan in organic solvents.

Keywords: Chitosan; Trimethylsilylation; Solubilization; Chemical modification; Acetylation

1. Introduction

Chitin is an abundant and easily accessible biopolymer probably suitable for developing a novel type of materials with various advanced functions, but only a limited amount is currently being used primarily because of the inherent intractable nature. Structural modifications would lead to practical applications in many fields such as medicine, cosmetics, agriculture, food processing, water treatment, support for enzymes, and membrane and chromatographic separation (Goosen, 1997; Kurita, 1997, 2001; Muzzarelli, 1977; Roberts, 1992). Compared to chitin having acetylated amino groups, chitosan should have the advantage in chemical manipulations due to the presence of free amino groups. Chitosan, however, shows poor affinity for organic solvents, which has made the controlled reactions difficult.

Some efforts have been done to impart solubility to chitosan, and phthaloylation has proved effective (Kurita, Ikeda, Yoshida, Shimojoh, & Harata, 2002; Kurita, Uno, Saito, & Nishiyama, 2000a; Nishimura, Kohgo, Kurita, & Kuzuhara, 1991). The resulting phthaloylated chitosan is soluble in common solvents and thus useful for preparing tailored derivatives with well-defined structures (Kurita, Akao, Yang, & Shimojoh, 2003a; Kurita, Kojima, Nishiyama, & Shimojoh, 2000b; Kurita, Shimada, Nishiyama, Shimojoh, & Nishimura, 1998; Nishimura et al., 1993, 1998; Nishiyama et al., 2000; Ouchi, Nishizawa, & Ohya, 1998; Rout, Barman, Pulapura, & Gross, 1994; Rout, Pulapura, & Gross, 1993a,b). Trimethylsilylation of chitosan may be another way of solubilization as suggested from the silylation of cellulose (Harmon, De, & Gupta, 1973; Mormann & Demeter, 1999), amylose (Horton & Lehmann, 1978), chitin (Kurita, Hirakawa, & Nishiyama, 1999), and dextran (Nouvel, Dubois, Dellacherie, & Six, 2003). Unlike phthaloylation, the silylation will possibly leave the amino group available for modification reactions, and a preliminary study showed the approach highly promising (Kurita, Hirakawa, Aida, Yang, & Nishiyama, 2003b). Here we report the results of the detailed study on the trimethylsilylation of chitosan and some properties including the solubility and reactivity characteristics of the product.

2. Experimental

2.1. General

IR spectra were recorded on a Shimadzu FTIR-8900 instrument by the KBr method. Elemental analysis was

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carried out with a Perkin Elmer 2400 instrument. Gel permeation chromatography (GPC) was conducted 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; standard, pullulans). The degree of deacetylation was determined by conductometric titration with a TOA conductivity meter CM-40S. Solvents were purified by distillation in usual manners and stored over molecular sieves. Chemicals were of reagent grade and used as received.

2.2. Chitosans

α-Chitin isolated from shrimp shells was pulverized and treated with 40% aqueous sodium hydroxide at 110 °C for 4 h in nitrogen. The deacetylated product was filtered and washed with deionized water. The alkaline treatment was repeated two more times to give chitosan (chitosan (α)) with a degree of deacetylation of 1.0. The number average and weight average molecular weights (M_n and M_w) determined by GPC were 93,300 and 411,000, respectively.

β-Chitin from squid pens was treated with 40% sodium hydroxide at 80 °C in nitrogen two times to give fully deacetylated chitosan (chitosan (β)) (Kurita et al., 1993a). GPC measurement indicated the M_n and M_w to be 81,200 and 230,000.

2.3. Trimethylsilylation of chitosan

Chitosan prepared from β-chitin (0.100 g, 0.62 mmol pyranose) was pulverized and added to 10 ml of pyridine. The dispersion was heated at 100 °C for 24 h and allowed to cool to room temperature. Hexamethyldisilazane (1.00 g, 6.2 mmol), chlorotrimethylsilane (0.94 g, 6.2 mmol), and 4-dimethylaminopyridine (DMAP; 0.38 g, 3.1 mmol) were added, and after being heated at gentle reflux for 48 h with vigorous stirring, the mixture was poured into 150 ml of acetone. The precipitate was filtered, washed with water, and dried in vacuo to give 0.104 g of a pale tan powder. The degree of substitution (ds) was calculated to be 2.90 from the C/N value of elemental analysis. IR (KBr): ν 3400 (NH), 2957 (CH), 2887 (CH), 1254 ($SiCH_3$), 1150–1000 (pyranose), and 843 cm^{-1} ($SiCH_3$).

2.4. Desilylation to regenerate chitosan

To 20 ml of a mixed solvent of methanol/water/acetic acid (2:1:1) was added 0.050 g of silylated chitosan (ds 2.50), and the mixture was stirred at room temperature for 24 h. The resulting solution was freeze-dried, and the solid was dissolved in 10 ml of water. Aqueous sodium hydroxide (0.1 mol/l) was added to the solution to adjust the pH at 9.0. The precipitate was collected by filtration, washed with water and then with methanol, and dried to give 0.021 g (89%) of chitosan as a pale tan solid. The IR spectrum was identical with that of the starting chitosan.

2.5. Acetylation of trimethylsilylated chitosan

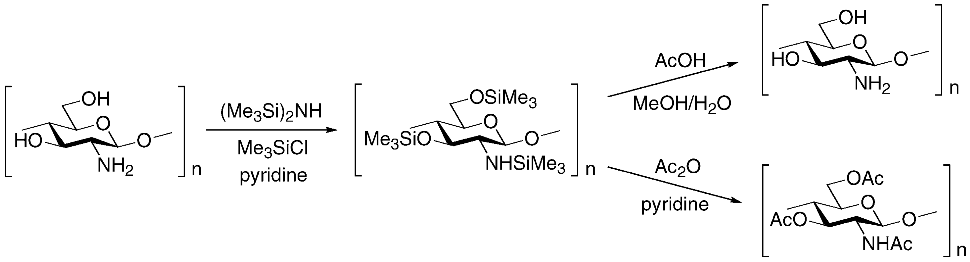
Trimethylsilylated chitosan (ds 2.82, 0.10 g, 0.275 mmol pyranose) was dispersed in 10 ml of pyridine, and 2.59 ml (2.81 g, 27.5 mmol) of acetic anhydride and 0.051 g (4.13 mmol) of DMAP were added. The mixture was stirred at room temperature for 16 h in nitrogen and added to ice water dropwise. The fibrous precipitate was collected by centrifugation, washed thoroughly with deionized water, and dried to give 68 mg of acetylated chitosan as a pale tan powdery material. The ds value was calculated to be 3.05 from the C/N value of elemental analysis. IR (KBr): ν 3380 (OH and/or NH), 1744 (ester C=O), 1670 (amide I), 1545 (amide II), 1236 (ester C–O–C), and 1150–1000 cm^{-1} (pyranose).

3. Results and discussion

3.1. Trimethylsilylation of chitosan

Trimethylsilylation was examined in detail using chitosans from α- and β-chitins (chitosan (α) and chitosan (β)) to establish an efficient and reliable procedure. Chitosan was first heated in pyridine at 100 °C to enhance affinity for the solvent and treated with a mixture of hexamethyldisilazane and chlorotrimethylsilane (Scheme 1). The reaction proceeded under heterogeneous conditions, and the product was isolated in acetone as an off-white to light tan powdery material.

Both chitosans were silylated slowly at room temperature, but as summarized in Table 1, chitosan (β) exhibited



Scheme 1.

Table 1
Trimethylsilylation of chitosan

Chitosan	Solvent	DMAP/pyranose (mol/mol)	Temp (°C)	Time (h)	Yield (%)[a]	ds for TMS[b]
Chitosan (α)	Pyridine	–	rt	24	46	0.19
Chitosan (α)	Pyridine	–	rt	72	44	0.33
Chitosan (α)	Pyridine	–	100	24	88	2.50
Chitosan (β)	Pyridine	–	rt	24	46	0.49
Chitosan (β)	Pyridine	–	rt	48	49	1.14
Chitosan (β)	Pyridine	–	70	24	51	1.14
Chitosan (β)	Pyridine	–	100	24	83	2.64[c]
Chitosan (β)	Pyridine	–	100	48	85	2.69
Chitosan (β)	Pyridine	5	100	48	82	2.82
Chitosan (β)	Pyridine	5	100	48	62	0.74[d]
Chitosan (β)	Pyridine	5	reflux	48	45	2.90[e]
Chitosan (β)	Pyridine	5	reflux	72	66	2.73
Chitosan (β)	Quinoline	5	120	48	69	2.40
Chitosan (β)	Quinoline	5	120	72	77	2.46
Chitosan (β)	Sulfolane	5	100	72	59	0.08

Chitosan, 0.10 g; solvent, 10 ml; silylating agents, 10 equivalents.

[a] Calculated on the basis of the ds value.

[b] Degree of substitution for trimethylsilyl group calculated from the C/N value of elemental analysis.

[c] Calcd for $(C_{12}H_{27}NO_4Si_2)_{0.46}(C_{15}H_{35}NO_4Si_3)_{0.64}\cdot 0.8H_2O$: C, 45.66; H, 9.28; N, 3.83. Found: C, 45.73; H, 9.17; N, 3.83.

[d] Bromotrimethylsilane was used instead of chlorotrimethylsilane.

[e] Calcd for $(C_{12}H_{27}NO_4Si_2)_{0.10}(C_{15}H_{35}NO_4Si_3)_{0.90}\cdot 0.5H_2O$: C, 46.53; H, 9.35; N, 3.69. Found: C, 46.53; H, 9.16; N, 3.69.

higher reactivity than chitosan (α). The similar reactivity difference was also observed in the phthaloylation of the two types of chitosans (Kurita, Tomita, Tada, Nishimura, & Ishii, 1993b). The high reactivity of chitosan (β) would be attributable most likely to the amorphousness in contrast to the crystalline structure of chitosan (α) (Kurita et al., 1993a).

At 70 °C, the ds was still around 1 after 24 h, though silylation of chitin was complete in 16 h at this temperature (Kurita et al., 1999). The reaction was facile at 100 °C, and the ds increased to 2.3 in 7 h. The difference in reactivity between the two chitosans was not very evident at 100 °C, but chitosan (β) was still more reactive, the ds values of the derivatives being higher by about 0.1.

In the presence of DMAP, the ds was improved to some extent, about 0.15, although the influence of the amount of the catalyst was not so pronounced from 1 to 7 equivalents as shown in Fig. 1. The results in Table 1 confirm that a higher temperature (reflux) or a prolonged time (72 h) was not effective to further improve the substitution. The ds reached 2.9 under appropriate conditions.

Attempts to raise the ds by conducting the reaction at 120 °C in quinoline were not successful. Sulfolane was not suitable as shown in Table 1. Bromotrimethylsilane was also used in place of chlorotrimethylsilane, but much less effective; the ds was 0.74 with bromotrimethylsilane in comparison to 2.82 with chlorotrimethylsilane under similar conditions. Prolonged heating at elevated temperatures caused discoloration of the product to brown, and the reaction in pyridine at 100 °C for 48 h appeared to be suitable for attaining a high ds without heavy coloration.

Fig. 2 shows the IR spectra of the silylated products as compared with that of the original chitosan. In the course of silylation, the bands characteristic of Si–CH$_3$ at 1254 and 843 cm^{-1} and those of C–H at 2880–2957 cm^{-1} became strong, and the broad band at 3400 cm^{-1} due to OH and NH$_2$ became weak. The structure of the product was also supported by elemental analysis; all the products gave satisfactory analysis data for C, H, and N within ± 0.3% of theoretical values. Examples are given in the footnote of Table 1.

3.2. Properties of silylated chitosan

The silylated chitosan was expected to show high solubility, and the results of a qualitative solubility test in

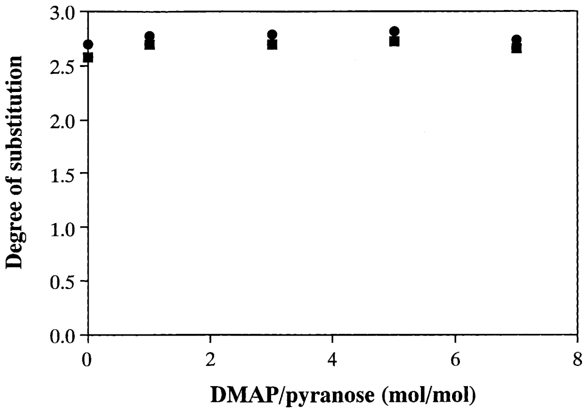


Fig. 1. Influence of the amount of DMAP on the trimethylsilylation of chitosan (α, ■) and chitosan (β, ●) with 10 equivalents of silylating agents in pyridine at 100 °C for 48 h.

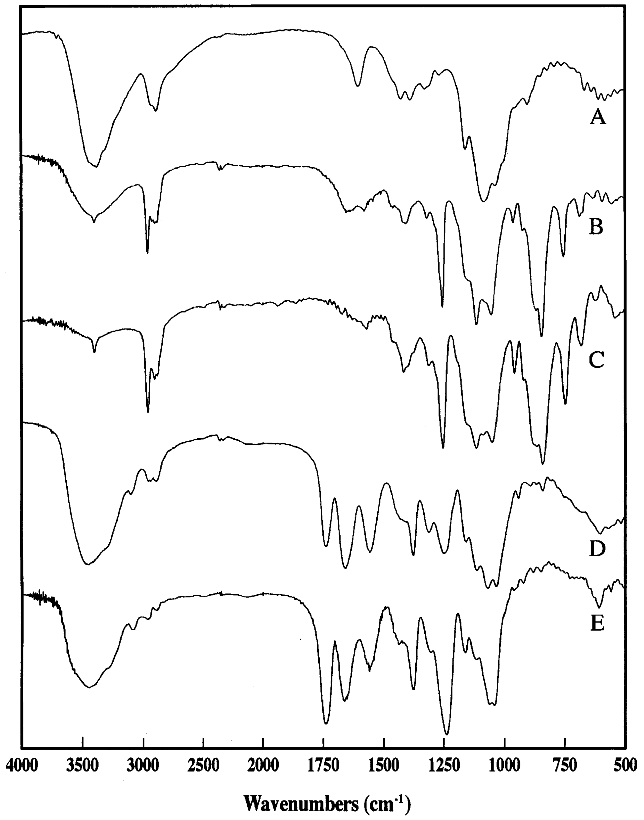


Fig. 2. IR spectra of chitosan (A), trimethylsilylated chitosan with ds 2.40 (B), trimethylsilylated chitosan with ds 2.90 (C), acetylated chitosan with ds 2.55 (D), and acetylated chitosan with ds 3.17 (E).

excess solvents are listed in Table 2. The affinity for organic solvents was improved on trimethylsilylation, and the derivative with ds 2.8–2.9 was almost soluble in pyridine leaving a small amount of transparent swollen gels. It was partially soluble and highly swelled in dimethyl sulfoxide. Low boiling common solvents such as acetone, methanol, and chloroform swelled it moderately.

The resulting trimethylsilyl ether bond was rather stable in neutral and alkaline solutions, and in saturated aqueous sodium hydrogen carbonate as well as water, no appreciable change in the IR spectrum was observed after 24 h at room temperature. However, the bond was cleaved readily in an acidic solution, and for instance, a dispersion of silylated chitosan in a mixed solvent of methanol/water/acetic acid (2:1:1) became a clear solution at room temperature in 1 h, indicating facile deprotection (Scheme 1). The regenerated chitosan could be recovered almost quantitatively.

Table 2
Solubility

	Acetone	THF	Pyridine	DMSO	DMAc
Chitin	−	−	−	−	−
Chitosan	−	−	−	−	−
TMS-chitosan[a]	±	±	+	±	±

THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; DMAc, *N*,*N*-dimethylacetamide; +, almost soluble; ±, partially soluble or swelled; −, insoluble.

[a] Trimethylsilylated chitosan (ds 2.82).

3.3. Acetylation of trimethylsilylated chitosan

Improved solubility of silylated chitosan and the labile nature of O–SiMe$_3$ and N–SiMe$_3$ linkages suggested that the product would be promising as a precursor for chemical modifications. Silylated chitosan was therefore subjected to acetylation as a typical modification reaction to evaluate the possibility as a reaction precursor (Scheme 1).

As summarized in Table 3, when two types of the original chitosans were treated with acetic anhydride in pyridine at room temperature for 48 h in the presence of DMAP, chitosan (β) again underwent acetylation more easily, and the ds was 2.22 in contrast to 1.53 for chitosan (α). As anticipated, silylation markedly enhanced the reactivity, and a ds of 2.55 was achieved even in the absence of the catalyst. On addition of DMAP, the ds increased to 2.85 in 6 h and around 3.0 in 16 h. A prolonged reaction time over 16 h resulted in the formation of derivatives with ds slightly above 3.0. All the products gave satisfactory elemental analysis data, and examples are given in the footnote of Table 3.

Fig. 2 includes the IR spectra of the acetylated products that show distinctive ester (1744 and 1236 cm^{-1}) and amide bands (1670 and 1545 cm^{-1}). However, the ester bands of the products with ds 2–2.5 were still moderate while the amide bands were strong. Moreover, a broad band ascribable to the OH was observed at around 3440 cm^{-1}, though it became weak for the product with ds around 3.0. These results imply that the preferential acetylation site is the amino group in the silylated chitosan,

Table 3
Acetylation of trimethylsilylated chitosan

Chitosan	DMAP/pyranose (mol/mol)	Temp (°C)	Time (h)	Yield (%)[a]	ds for Ac[b]
Chitosan (α)	1.5	rt	48	27	1.53
Chitosan (β)	1.5	rt	48	32	2.22
TMS-chitosan	–	rt	48	60	2.55[c]
TMS-chitosan	1.5	rt	6	79	2.85
TMS-chitosan	1.5	rt	16	86	3.05[d]
TMS-chitosan	1.5	rt	24	92	3.19
TMS-chitosan	1.5	rt	48	56	3.16
TMS-chitosan	1.5	rt	72	53	3.17
TMS-chitosan	1.5	50	48	90	3.20

Trimethylsilylated chitosan (ds 2.82), 0.10 g; pyridine, 10 ml, acetic anhydride, 2.59 ml.

[a] Calculated on the basis of the ds value.

[b] Degree of substitution for acetyl group calculated from the C/N value of elemental analysis.

[c] Calcd for $(C_{10}H_{15}NO_6)_{0.45}(C_{12}H_{17}NO_7)_{0.55}\cdot 0.6H_2O$: C, 47.76; H, 6.25; N, 5.02. Found: C, 47.69; H, 6.26; N, 5.01.

[d] Calcd for $(C_{12}H_{17}NO_7)_{0.95}(C_{14}H_{19}NO_8)_{0.05}\cdot 0.3H_2O$: C, 49.30; H, 6.05; N, 4.75. Found: C, 49.31; H, 6.25; N, 4.75.

and *N*,*N*-diacetylation could proceed along with *O*-acetylation even under such mild conditions. The reaction behavior of silylated chitosan is quite interesting and is now being studied in detail.

4. Conclusions

Trimethylsilylation of chitosan proceeded with a mixture of hexamethyldisilazane and chlorotrimethylsilane in pyridine at 100 °C to give a derivative with ds up to 2.9, conditions more severe than those for trimethylsilylation of chitin. DMAP was effective as a catalyst only to a limited extent. The silylation proved to greatly enhance the reactivity as well as solubility in organic solvents, and it is noteworthy that even *N*,*N*-diacylation could be possible at room temperature. The silylated chitosan obtained here will therefore be useful as a reaction precursor for substitution both at hydroxy and amino groups to synthesize highly substituted chitosan derivatives under mild conditions.

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