New Selectively *N*-Substituted Quaternary Ammonium Chitosan Derivatives

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ABSTRACT: New chitosan derivatives were synthesized by *N*-selective introduction of quaternary ammonium-type side chains without protection of hydroxy groups, although direct quaternization of the amino group of chitosan using alkyl halides under alkaline conditions introduced alkyl groups not only to the amino group but also to the hydroxy groups. The reductive *N*-alkylation reaction of chitosan with quaternary ammonium-type aldehydes, such as 4-formyl-*N*-methylpyridinium iodide and [3-(4-formylphenoxy)propyl]trimethylammonium iodide, followed by anion-exchange reaction gave partially *N*-*p*-(*N*-methylpyridinio)methylated chitosan chloride (*N*-MP [Cl[−]]) and partially *N*-4-[3-(trimethylammonio)propoxy]benzylated chitosan chloride (*N*-TMPB [Cl[−]]), respectively. From the viewpoint of utilization as antistatic material for functional textile, the electric resistance of newly prepared derivatives were investigated. In comparison with known quaternary ammonium chitosan derivatives (Q-CDs), such as 96% deacetylated chitin hydrochloride (DAC-96·HCl), partially *O*-methyl-*N*-trimethylated chitosan chloride (*O*-Me-*N*-TM [Cl[−]]), and partially *N*,*O*-(2-hydroxy-3-trimethylammonio)propylated chitosan chloride (*N*,*O*-HTMP [Cl[−]]), *N*-MP [Cl[−]] showed the lowest specific resistance (ρ). *N*-TMPB [Cl[−]] whose ρ was comparable with that of *N*-TM [Cl[−]] showed lower water content compared with known Q-CDs noted above. Newly prepared Q-CDs thus appear as promising biocompatible antistatic materials.

KEY WORDS Quaternary Ammonium Chitosan Derivative / *N*-Selective Substitution / Reductive *N*-Alkylation / Electric Resistance / Ionic Conduction /

Chitosan is a β -(1→4)-linked natural polysaccharide composed of 2-amino-2-deoxy- β -D-glucopyranose (D-glucosamine) residues (Figure 1). Chitosan has been industrially obtained by deacetylation of chitin, a polysaccharide made up of β -(1→4)-linked 2-acetamido-2-deoxy- β -D-glucopyranose units. Chitin and chitosan have gained increasing attention due to biocompatibility¹ and antimicrobial activity.² The degree of deacetylation (DDA) of partially deacetylated chitin (DAC) can be controlled by the deacetylating conditions.³ To use biocompatible natural polysaccharides as antistatic materials for the functional textile, we previously studied the specific resistance of various chitosan derivatives in the presence or absence of dopants.⁴ Ionic substituent groups, such as carboxymethyl group, sulfate group, and quaternary ammonium group, provide ionic carriers to reduce the specific resistance (ρ) without the addition of dopant,⁴ and thus we chose relatively neutral ionic groups, ammonium-type substituents, in this study. As shown in Figure 2, transformation of chitosan which was

96% deacetylated chitin (DAC-96) to the ionic derivative DAC-96·HCl reduced the specific resistance (ρ). But chitosan hydrochloride (DAC-96·HCl) showed high water content (16%) responsible for the unstable and acidic characters of hygroscopic DAC-96·HCl. To improve these disadvantageous characters for the utilization as textile materials, various quaternary ammonium chitosan derivatives (Q-CDs) were prepared, and the effects of the chemical and structural properties of *N*-substituents on the water content and ρ of chitosan derivatives were investigated. Compared with known ammonium-type chitosan derivatives, new stable chitosan derivatives which have bulky quaternary ammonium side chains showed lower ρ , although the effect of the new Q-CDs on human skin has not been probed yet.

EXPERIMENTAL

Materials and Installation

DAC-96 (Chitosan 10B, Lot. No. PJ16-M, powder, DDA = 96%, weight average molecular weight ($\bar{M}_w$) = 189000) was purchased from Funakoshi Co., Ltd. Unless otherwise noted, other reagents were purchased from Wako Pure Chem. Ind., Ltd. and Aldrich Chem. Co., Inc. DDA were determined by the NMR (270 MHz) and IR method.⁵ Molecular weights of water soluble chitosan derivatives were determined by GPC ($\bar{M}_w$: calculated on the basis of pullulan calibration). The content of Cl[−] was determined by elemental analysis.

Preparation of *N*-Butylated Chitosan Derivatives

As previously described,⁴ *N*-butylated DAC-90 (*N*-Bu-DAC-90) was prepared by Muzzarelli's method.⁶ *N*-Bu-DAC-90·HCl salt was obtained by the dialysis of a 0.1 M aqueous HCl solution (100 mL) of *N*-Bu-DAC-90 (24 mmol as hexosamine residue) followed by lyophilization.

DDA: Degree of Deacetylation

Chitin: DDA < 5%

Chitosan: DDA > 95%

DAC: Partially Deacetylated Chitin

Figure 1. Chitin and chitosan.

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Figure 2. Design of new quaternary ammonium chitosan derivatives. ρ : specific resistance (Ω cm) ; measurement conditions : 20°C, RH = 40%.

Preparation of Quaternary Ammonium Chitosan Derivatives (Q-CD)

Degree of substitution (DS), $\bar{M}_w$, and spectral data were given in Table I. DS was determined by the ^1H NMR analysis.

Partial *O*-Methyl-*N*-trimethylation of Chitosan. *N*-Quaternization and *O*-alkylation of DAC were carried out by modification of Domard's method.⁷ DAC-96 (2.0 g, 12 mmol as hexosamine residue) was dispersed in *N*-methyl-2-pyrrolidone (100 mL) overnight at room temperature. An aqueous solution (10 mL) of NaOH (1.2 g, 30 mmol) and NaI (2.5 g, 16.7 mmol) was added to the DAC-96 suspension and MeI (25.6 g, 0.18 mol, 15 equivalent relative to the hexosamine residue) was added. After stirring at 36°C for 8 h, the addition of ethanol to the reaction mixture produced a precipitate, which was redissolved in water (20 mL). This aqueous solution was treated by anion-exchange resin (40 mL, Amberlite IR-45, Cl^- type), dialyzed, and lyophilized to give *O*-methyl-*N*-trimethyl-DAC-99 chloride as a colorless powder (*O*-Me-*N*-TM [Cl^-] : 1.9 g, 80% yield).

Partially *N*-*p*-(*N*-Methylpyridinio)methylated Chitosan Chloride (*N*-MP [Cl^-]). After mixing 4-pyridinecarboxaldehyde (4.0 g, 38 mmol) and MeI (10 g, 57 mmol), removal of excess MeI under reduced pressure and washing with CHCl_3 and Et_2O gave 4-formyl-*N*-methylpyridinium iodide (**1**), used for reductive *N*-alkylation of DAC-96 without further purification. A methanol solution (100 mL) of **1** (3.4 g, 12 mmol) was added to DAC-96 (2.0 g, 12 mmol as glucosamine residue) dissolved in 2 wt% aqueous acetic acid (100 mL). After 30 min, NaBH_4 (0.50 g, 13 mmol) was added portion-wise to the reaction mixture for a period of 1 h at room temperature. pH of the reaction mixture was raised to 10 by adding 1 M NaOH, and the precipitate formed was washed with ethanol and dialyzed. Lyophilization of the suspension gave *N*-MP [OH^-] (yellowish sponge, 2.1 g, 95% yield). After *N*-MP [OH^-] dissolved in 0.1 M HCl (40 mL) containing 5% NaCl was dialyzed against 5%

NaCl solution and then against distilled water, lyophilization of the solution gave partially *N*-*p*-(*N*-methylpyridinio)methylated 96% deacetylated chitin chloride (*N*-MP [Cl^-]) (yellowish sponge).

Partially *N*-4-[3-(Trimethylammonio)propoxy]benzylated Chitosan Chloride (*N*-TMPB [Cl^-]). [3-(4-formylphenoxy)propyl]trimethylammonium iodide (**2**) was prepared from MeI and 4-[3-(dimethylamino)propoxy]benzaldehyde by the same method as for the preparation of **1**. By the method for the synthesis of *N*-MP [OH^-] and *N*-MP [Cl^-], *N*-TMPB [OH^-] (1.8 g, 90% yield) was prepared from **2** and DAC-96, and converted to partially *N*-4-[3-(trimethylammonio)propoxy]benzylated 96% deacetylated chitin chloride (*N*-TMPB [Cl^-]) (colorless sponge).

Partially *N*,*O*-[2-Hydroxy-3-(trimethylammonio)]propylated Chitosan (*N*,*O*-HTMP). By the modified method of Amiya *et al.*,⁸ the addition of 1 M NaOH to a 2 wt% aqueous acetic acid (200 mL) solution of DAC-96 (2.0 g, 12 mmol as hexosamine residue) increased the pH of the solution to 10 and produced the precipitate, which was separated and suspended in 3.7 M NaOH (50 mL). After cooling in an ice bath, a 50% aqueous solution of 3-chloro-2-hydroxypropyltrimethylammonium chloride (2.0 g, 12 mmol) was added to the suspension. The reaction mixture was stirred at 0°C for 1 h and then at 40°C for 18 h. Dialysis of the reaction mixture followed by lyophilization gave *N*,*O*-HTMP [OH^-] (colorless powder, 2.4 g, 95% yield), which was transformed to partially *N*,*O*-[2-hydroxy-3-(trimethylammonio)]propylated 99% deacetylated chitin chloride (*N*,*O*-HTMP [Cl^-]) by the same method as the preparation of *N*-MP [Cl^-].

Preparation of Tablets and Determination of Electric Resistance

The electric resistance of Q-CD was determined by the tablet method reported previously.⁴ Tablets (ca. 150 mg, 13 mm in diameter, and ca. 1 mm in thickness) of chitosan derivatives were prepared by direct compression at

Table I. Chitosan Derivatives

Sample	R	R ¹	DDA	DS ^a	Cl ^{-b}	$M_w^c \times 10^3$	IR	¹ H NMR
DAC-96	1	4 or 5	96	—	—	189	3500–3400 (NH ₂ , OH), 2900 (CH), 1650, 1320 (amide), and 1080 (C–O–C)	δ = 2.06 (0.12H, s, <i>N</i> -Ac), 3.2–4.5 (6H, m, C2–C6 protons), and 4.6–5.2 (1H, m, C1 proton)
<i>N</i> -Bu-DAC-90	1	4, 5, or 6	90	0.8	—	250	3500–3400 (NH ₂ , OH), 2900 (CH), 2850 (CH), 1650, 1320 (amide), and 1080 (C–O–C)	δ = 0.93 (2.6H, t, <i>J</i> = 7.3 Hz, CH ₃), 1.3–1.4 (1.6H, m, CH ₂), 1.7–1.8 (1.6H, m, CH ₂), 2.06 (0.3H, s, <i>N</i> -Ac), 3.3–4.1 (7.7H, m, C2–C6 protons and N-CH ₂), and 4.9–5.2 (1H, m, C1 proton)
<i>O</i> -Me- <i>N</i> -TM [Cl ⁻]	1 or 2	4, 5, or 7	99	DS (N) 0.4 DS (O) 0.3	0.9	104	3500–3400 (NH ₂ , OH), 2900 (CH), 1650, 1560 (amide), and 1080 (C–O–C)	δ = 2.06 (0.03H, s, <i>N</i> -Ac), 3.12 (3.2H, s, N ⁺ Me ₃), 3.2–4.5 (6.8 H, m, C2–C6 protons and <i>O</i> -Me), and 4.6–5.2 (1H, m, C1 proton)
<i>N,O</i> -HTMP [Cl ⁻]	1 or 3	4, 5, or 10	99	DS (N+O) 0.29	0.6	251	3500–3400 (NH ₂ , OH), 2900 (CH), 1630, 1490 (amide), 1080 (C–O–C), and 905 (CH)	δ = 2.06 (0.03H, s, <i>N</i> -Ac), 2.8–2.9 (3.2H, m, C-CH ₂ -N ⁺), 3.20 (3.2H, s, N ⁺ Me ₃), 3.4–4.2 (7.1 H, m, C2–C6 protons and <i>N</i> , <i>O</i> -CH ₂ -CH(OH)-), and 4.4–4.6 (1H, m, C1 proton)
<i>N</i> -MP [Cl ⁻]	1	4, 5, or 8	96	0.16	0.7	—	3500–3400 (NH ₂ , OH), 3100 – 2890 (CH), 1630, 1510 (amide), and 1090 (C–O–C)	δ = 2.06 (0.12H, s, <i>N</i> -Ac), 3.19 (0.5H, s, N ⁺ Me), 3.4–4.2 (6H, m, C2–C6 protons), 4.43 (0.3 H, s, <i>N</i> -CH ₂ -C ₅), 4.6–5.2 (1H, m, C1 proton), and 8.2–8.9 (0.6H, m, C ₅ H ₄ N ⁺)
<i>N</i> -TMPB [Cl ⁻]	1	4, 5, or 9	96	0.14	1.0	350	3500–3400 (NH ₂ , OH), 2890 (CH), 1630, 1530, 1380 (C–N–C), and 1080 (C–O–C)	δ = 2.06 (0.12H, s, <i>N</i> -Ac), 2.2–2.3 (0.3H, m, C-CH ₂ -C), 3.16 (1.3H, s, N ⁺ Me ₃), 3.1–3.3 (1.0 H, m, C2 proton), 3.57 (0.3H, m, N ⁺ -CH ₂ -C), 3.7–4.1 (5H, m, C3–C6 protons), 4.20–4.25 (0.3H, m, <i>O</i> -CH ₂ -C), 4.46 (0.3H, s, N-CH ₂ -C ₆), 4.6–5.0 (1H, m, C1 proton), and 7.1–7.5 (0.6H, m, C ₆ H ₄)

^a DS(N): degree of *N*-trialkylated substitution; DS (O): degree of *O*-alkylated substitution; calculated by the ¹H NMR analysis. ^b Content: mole equivalent relative to hexosamine residue. ^c M_w , weight average molecular weight; GPC, Asahipak GS-510H, GS-310M, GS-220 H; 0.1 M AcOH/AcONa buffer containing 0.1 M NaCl (pH 4.8), calculated on the basis of pullulan calibration.

400 kg cm⁻² for 10 min. Electrodes on the tablets were made with silver paste as shown in Figure 3. The dry weight (W_0) of tablets was determined after drying under reduced pressure (10 mmHg) at 60°C for 24 h. The weight of a tablet was equilibrated at the prescribed temperature and relative humidity (*RH*) in an environmental chamber (LH-20, Nagano Science Ltd.). After determination of electric resistance (r/Ω), weight (W_t) and thickness (h /cm) of the tablets were determined. The water content ($H/\%$) was calculated by eq 1

$$H = 100(\Delta W/W_0) \quad (1)$$

where ΔW is increasing weight of tablet by water sorption ($W_t - W_0$ (mg)).

Figure 3. Chitosan tablet for measuring electric resistance. ■: Electrode of silver paste; d : electrode distance, 0.5 cm; h : thickness, 0.09–0.11 cm; w : width of electrode, 1.1 cm.

Scheme 1.

After charging by DC 100 V for 1 min, the electric resistance (r) of tablet was measured by an ohmmeter (Ultra Megohmmeter SM-8210, TOA Electronics Ltd.). Specific resistance (ρ) was calculated by eq 2

$$\rho = r(wh/d) = 1/\sigma \quad (2)$$

where ρ is specific resistance (Ω cm), w : width of electrode (cm), h : thickness of tablet (cm), d : electrode distance (cm), and σ : electric conductivity.

RESULTS AND DISCUSSION

Construction of New Chitosan Derivatives with Quaternary Ammonium-Type Side Chains

Because quaternization of the amino group of chitosan with alkyl halides is generally accompanied by alkylation reaction of the hydroxy groups,⁷ the reductive *N*-alkylation method⁶ was applied for the *N*-selective introduction of side chains already quaternized. As shown in Scheme 1, new chitosan derivatives *N*-MP and *N*-TMPB were synthesized by reductive *N*-alkylation of DAC-96 with quaternary ammonium compounds having an aldehyde function such as 4-formyl-*N*-methylpyridinium iodide (**1**) and [3-(4-formylphenoxy)propyl]trimethylammonium iodide (**2**), respectively. *N*-Selective introduction of the quaternary ammonium-type side chains was confirmed by ¹H NMR signals (δ 4.43 for *N*-MP and δ 4.46 for *N*-TMPB) corresponding to methylene groups attached to the amino group of glucosamine residue. Singlet peaks corresponding to the methyl protons of *N*-MP and *N*-TMPB were observed at 3.19 and 3.16 ppm, respectively. Substitution (*DS*) of *N*-alkylation was estimated by the ¹H NMR analysis of the side chain protons and chitosan chain protons shown in Table I. When exposed to ultraviolet light of wavelength 350 nm, *N*-MP dissolved in dilute CH₃COOH with fluoresced light blue color (λ_{em} 450 nm). As for the water solubility of these Q-CDs, [Cl[−]] type salts were soluble in distilled water, al-

Table II. Specific Resistance (ρ) of Chitosan Derivatives

Run	Sample	Water Content ^a	$\log \rho$ ^a
		%	
1	DAC-96	6	9.3
2	DAC-96·HCl	16	6.4
3	<i>N</i> -Bu-DAC-90	5	11.0
4	<i>N</i> -Bu-DAC-90·HCl	5	8.2
5	<i>O</i> -Me- <i>N</i> -TM [Cl [−]]	11	6.8
6	<i>N</i> , <i>O</i> -HTMP [OH [−]]	5	9.1
7	<i>N</i> , <i>O</i> -HTMP [Cl [−]]	9	5.8
8	<i>N</i> -MP [OH [−]]	1	9.5
9	<i>N</i> -MP [Cl [−]]	10	5.2
10	<i>N</i> -TMPB [OH [−]]	4	9.1
11	<i>N</i> -TMPB [Cl [−]]	6	6.5

^a Measurement conditions: 20°C, RH = 40%.

though [OH[−]] types were insoluble. Hence, the water content (H%) of these Q-CDs of [OH[−]] type was much less than that of DAC-96·HCl in Table II. Compared with chloride ion in Q-CD [Cl[−]], hydroxide ion in Q-CD [OH[−]] binds chitosan chains very tightly by the formation of hydrogen bonds, resulting in a small water sorption of Q-CD [OH[−]].

Electric Resistance of Quaternary Ammonium Chitosan Derivatives (Q-CDs)

Based on the study on the simple and swift estimation of the specific resistance (ρ) of various chitosan derivatives using tablets made by direct compression of chitosan samples,⁴ the electric resistance of Q-CD [OH[−]] type and [Cl[−]] salt type was determined. Specific resistance (ρ) and water content of various Q-CDs are shown in Table II. Comparison of Runs 6, 8, and 10 with Runs 7, 9, and 11 revealed that the ρ values of Q-CDs decreased by transformation of [OH[−]] type into [Cl[−]] salt type, and water content of Q-CD with Cl[−] as a counter ion (Q-CD [Cl[−]]) was larger than that of Q-CD [OH[−]]. Because sorption of a large amount of water causes many prob-

Figure 4. Relationship between temperature (T) and electric conductivity (σ) of (◆) O -Me- N -TM [Cl^-], (●) N , O -HTMP [Cl^-], (■) N -MP [Cl^-], and (▲) N -TMPB [Cl^-]; measurement conditions: 20–60°C (293–333 K), $RH = 40\%$.

lems based on the chemical and structural instability, the high water-sorptive property of Q-CD is not suitable for the application as functional textile materials. To suppress water sorption, a hydrophobic group (butyl group) was introduced to the chitosan chain. Although N -Bu-DAC-90·HCl showed low water content value, ρ remained at almost same level as that of DAC-96 as shown in Figure 2. Quaternization of the amino group of DAC-96 with the alkyl group of smallest bulkiness (methyl group) gave O -Me- N -TM [Cl^-] which showed low ρ and high water content. Also N , O -HTMP [Cl^-] which has a hydrophilic quaternary ammonium side chain showed low ρ and high water content (Run 7). New chitosan derivatives (N -MP and N -TMPB) with aromatic quaternary ammonium side chains were thus studied to decrease water content and ρ . Compared with DAC-96·HCl in Run 2, N -MP [Cl^-] (Run 9) had less water content. p - N -Methylpyridiniummethyl group in N -MP [Cl^-] was very effective for decreasing ρ and N -MP [Cl^-] showed the lowest specific resistance. N -TMPB [Cl^-] (Run 11), which has a hydrophobic quaternary ammonium side chain, suppressed water sorption keeping ρ almost same as that of DAC-96 doped with HCl. Newly synthesized Q-CDs thus appear promising biocompatible antistatic materials which needing no dopants such as acids and metal salts.⁹

Ionic Conductivity

Because $\ln \sigma$ of Q-CDs shown in Figure 4, was proportional to T^{-1} ,¹⁰ these derivatives were found to have ionic conductivity. Activation energies (E_a) of Q-CDs were calculated from the slopes of the liner plots of $\ln \sigma$ vs. T^{-1} .¹⁰ N -MP [Cl^-], N , O -HTMP [Cl^-], N -TMPB [Cl^-], and O -Me- N -TM [Cl^-] gave 30, 43, 72, and 94 kJ mol⁻¹ of E_a values, respectively. Therefore, N -MP [Cl^-], N , O -HTMP [Cl^-], and N -TMPB [Cl^-] which have small E_a provide the ionic carrier more effectively than O -Me- N -TM [Cl^-]. There was not so much difference in the specific resistance (ρ) of Runs 5, 7, 9, and 11 in Table II, even though substitution (DS) of N , O -HTMP [Cl^-] (0.3) and O -Me- N -TM [Cl^-] (0.4) was 2 times larger than those of N -MP [Cl^-] (0.16) and N -TMPB [Cl^-] (0.14). These results mean that N -selectively substituted Q-CDs (N -MP [Cl^-], N -TMPB [Cl^-]) whose hydroxy groups are free, are superior antistatic materials compared with N , O -substituted Q-CDs such as N , O -HTMP [Cl^-] and O -Me- N -TM [Cl^-].

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