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Comb-shaped chitosan derivatives having oligo(ethylene glycol) side chains

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Summary

Monoaldehydes have been synthesized from tri- and tetra(ethylene glycol) monosubstituted derivatives and introduced into chitosan by the reductive alkylation technique to give comb-shaped polysaccharide hybrids. The reaction of chitosan with the aldehydes in the presence of sodium cyanoborohydride proceeded efficiently to give chitosan derivatives having oligo(ethylene glycol) side chains at the amino groups. The products were characterized by high affinity for organic solvents as well as water in sharp contrast to the original chitin and chitosan. They showed significant adsorption capacity toward metal cations.

Introduction

Polysaccharides are natural polymers indispensable in many fields owing to their unique structures quite different from those of synthetic polymers. Of many kinds of polysaccharides, chitin and the deacetylated form, chitosan, are particularly interesting because of their distinctive physical properties and biological functions associated primarily with the presence of amino groups (1,2). Much attention has thus been paid to chitin to develop practical applications, but due to the lack of solubility it still remains an unutilized biomass resource.

Although modification reactions of chitin and chitosan are usually accompanied by some difficulties under heterogeneous conditions, organosoluble precursors have proved to be useful for preparing derivatives with well-defined structures under mild conditions (3,4). However, chitosan has free amino groups and has a substantial advantage over chitin in modifications. Reductive alkylation is a typical example of reactions of chitosan, and N-substituted chitosan derivatives were obtained from reducing sugars (5), aldehydes (6,7), or ketones (8). The reaction is important in that it affords derivatives having substituents only at the amino groups of this multifunctional polymer. Moreover, the N-substituted chitosans generally show better solubility than chitosan. Quite recently, poly(ethylene glycol) chains were introduced into chitosan by a similar procedure, and the products gave stable mixtures with an acrylic emulsion (9). We have examined the possibility of introducing side chains of the same length by reductive alkylation to prepare comb-shaped chitosan derivatives with well-defined homogeneous structures. The products with oligo(ethylene glycol) branches are expected to exhibit much improved affinity for organic solvents as well as water, still retaining advantageous properties of chitosan. Here we report some preliminary results about the comb-shaped chitosan hybrids.

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Experimental

General

IR spectra were taken with a JASCO IR-700. ^{1}H NMR spectra were recorded on a JEOL JNM-GX270, tetramethylsilane being used as the internal standard. Elemental analysis was performed with a Perkin Elmer 2400. Degree of deacetylation was determined by conductometric titration with a conductivity meter TOA CM-40S. Metal cations were analyzed by atomic emission spectroscopy with a Kyoto Koken ICP-AES UOP-1 Mark II or by UV-VIS spectroscopy with a JASCO Ubest-30. Solvents were purified in usual manners and stored over molecular sieves. Chemicals were of reagent grade and used as received.

Chitosan

Chitin isolated from shrimp shells was purified by treating with 1 mol/L aqueous sodium hydroxide at 100 °C for 8 h and washing with deionized water. The degree of deacetylation was 0.09. Chitin (20.0 g) was then deacetylated with 500 mL of 40% sodium hydroxide at 110 °C for 4 h under nitrogen, and the product was washed with water until neutral to give chitosan with a degree of deacetylation of 0.90. To attain complete deacetylation, the chitosan was pulverized and deacetylated two more times by the same procedure to give 12.7 g of chitosan as a colorless powdery material. The degree of deacetylation was 1.00.

Synthesis of tri(ethylene glycol) monobenzoate

A solution of 75.1 g (0.50 mol) of tri(ethylene glycol) and 55.7 g (0.55 mol) of triethylamine in 300 mL of tetrahydrofuran was cooled in an ice-bath, and 70.3 g (0.50 mol) of benzoyl chloride was added dropwise over a period of 30 min so as to keep the reaction temperature below 5 °C. The mixture was stirred for 1 h with cooling and then at room temperature for 2 h. It was filtered, and the filtrate was concentrated under reduced pressure to give a pale yellow liquid. The liquid was chromatographed on silica gel with chloroform/toluene (8:2) to elute dibenzoate and then with chloroform for monobenzoate and unsubstituted tri(ethylene glycol). The dibenzoate and monobenzoate were further chromatographed several times to give pure products as colorless oils; the yields were 45.1 g (25.2%) and 51.5 g (40.4%), respectively.

Dibenzoate: IR (neat): v 2890 (CH_2), 1717 (ester C=O), 1283 (ester C-O-C), 1123 (ether C-O-C), and 714 cm^{-1} (phenyl). ^{1}H NMR ($CDCl_3$): δ 3.71 (s, 4H, central CH_2), 3.82 (t, 4H, Bz-O-$CH_2C\underline{H}_2$), 4.47 (t, 4H, Bz-O-$C\underline{H}_2CH_2$), 7.40 (t, 4H, phenyl *meta*-H), 7.52 (t, 2H, phenyl *para*-H), and 8.06 ppm (d, 4H, phenyl *ortho*-H).

Monobenzoate: IR (neat): v 3440 (OH), 2880 (CH_2), 1719 (ester C=O), 1276 (ester C-O-C), 1113 (ether C-O-C), and 716 cm^{-1} (phenyl). ^{1}H NMR ($CDCl_3$): δ 2.98 (broad s, 1H, OH), 3.60 (t, 2H, $C\underline{H}_2OH$), 3.70 (m, 6H, $C\underline{H}_2$), 3.82 (t, 2H, Bz-O-$CH_2C\underline{H}_2$), 4.47 (t, 2H, Bz-O-$C\underline{H}_2CH_2$), 7.40 (t, 2H, phenyl *meta*-H), 7.52 (t, 1H, phenyl *para*-H), and 8.06 ppm (d, 2H, phenyl *ortho*-H).

Synthesis of tetra(ethylene glycol) monobenzoate

Benzoylation of tetra(ethylene glycol) was carried out in a manner similar to that for tri(ethylene glycol). The dibenzoate and monobenzoate were obtained as colorless oils by repeated column chromatography, and the yields were 22.2% and 50.6%, respectively.

Dibenzoate: IR (neat): v 2880 (CH_2), 1721 (ester C=O), 1277 (ester C-O-C), 1112 (ether C-O-C), and 717 cm^{-1} (phenyl). ^{1}H NMR ($CDCl_3$): δ 3.67 (s, 8H, central CH_2), 3.83 (t, 4H, Bz-O-$CH_2C\underline{H}_2$), 4.44 (t, 4H, Bz-O-$C\underline{H}_2CH_2$), 7.41 (t, 4H, phenyl *meta*-H), 7.54 (t, 2H, phenyl *para*-H), and 8.04 ppm (d, 4H, phenyl *ortho*-H).

Monobenzoate: IR (neat): v 3430 (OH), 2880 (CH_2), 1719 (ester C=O), 1276 (ester C-O-C), 1112 (ether C-O-C), and 718 cm^{-1} (phenyl). ^{1}H NMR ($CDCl_3$): δ 3.17 (broad s, 1H, OH), 3.59 (t, 2H, $C\underline{H}_2$-OH), 3.67 (m, 10H, $C\underline{H}_2$), 3.83 (t, 2H, Bz-O-

$CH_2C\underline{H}_2$), 4.48 (t, 2H, Bz-O-$C\underline{H}_2CH_2$), 7.42 (t, 2H, phenyl *meta*-H), 7.54 (t, 1H, phenyl *para*-H), and 8.04 ppm (d, 2H, phenyl *ortho*-H).

Oxidation of tri(ethylene glycol) monomethyl ether

Tri(ethylene glycol) monomethyl ether (65.6 g, 0.40 mol) was dissolved in 800 mL of dry toluene, and 600 g of MnO_2, which had been dried at 150 °C for a few hours, was added. The mixture was heated under reflux for 48 h. After cooling, it was filtered several times to remove the solid completely. The filtrate was concentrated under reduced pressure, and the residual oil was chromatographed on silica gel with chloroform to give 48.7 g of a pale yellow liquid, which was confirmed to be a mixture of the aldehyde and starting material by TLC. The content of aldehyde was 97.4 mmol as determined by the oxime method (10), which is based on the titration of hydrogen chloride liberated from hydroxylamine hydrochloride as a result of reaction with the aldehyde, indicating that the yield was 24.4%.

IR (neat): v 2880 (CH_2), 1721 (aldehyde C=O), and 1112 cm^{-1} (ether C-O-C).

Oxidation of tri(ethylene glycol) monobenzoate

A mixture of 25.5 g (0.10 mol) of tri(ethylene glycol) monobenzoate and 250 g of MnO_2 in 700 mL of toluene was refluxed for 96 h. The mixture was worked up in a similar manner to give 19.7 g of a pale yellow liquid. Titration by the oxime method indicated that the content of aldehyde was 26.0 mmol. The yield was thus 26.0%.

IR (neat): v 2880 (CH_2), 1721 (ester and aldehyde C=O), 1276 (ester C-O-C), 1112 (ether C-O-C), and 717 cm^{-1} (phenyl).

Tetra(ethylene glycol) monobenzoate was oxidized by the same method as above, and the yield of the corresponding aldehyde was 40.5%.

Reductive alkylation of chitosan

A solution of 0.81 g (5.0 mmol of NH_2) of chitosan in 20 mL of 5% aqueous acetic acid was diluted with 20 mL of methanol. To the resulting viscous solution were added a solution of 2.50 g (5.0 mmol of aldehyde) of the oxidation product of tri(ethylene glycol) monomethyl ether in 10 mL of methanol and a solution of 1.33 g (20 mmol) of 95% $NaCNBH_3$ in 10 mL of methanol. The mixture became a soft gel immediately, which was stirred at room temperature for 18 h. It was dialyzed in deionized water for a week to give a clear solution. The solution was concentrated under reduced pressure and freeze-dried to give 1.19 g of a slightly yellow powdery material. Elemental analysis (found): C, 48.13%; H, 8.04%; N, 4.75%. The degree of substitution (ds) could be calculated from the C/N value based on these analytical data; the C/N value, 10.13, corresponds to ds 0.83.

IR (KBr): v 3430 (OH and NH), 2880 (CH_2), and 1200-1000 cm^{-1} (pyranose and C-O-C).

Adsorption of metal cations

In 10 mL of 5 mmol/L aqueous solution of a metal chloride was dispersed 10 mg of pulverized chitosan or a chitosan derivative, and the mixture was shaken at 25 °C for 24 h. The concentration of the supernatant was determined either by ICP-AES for Li^+, Na^+, and K^+, or by UV-VIS for Fe^{3+} and CU^{2+}.

Results and discussion

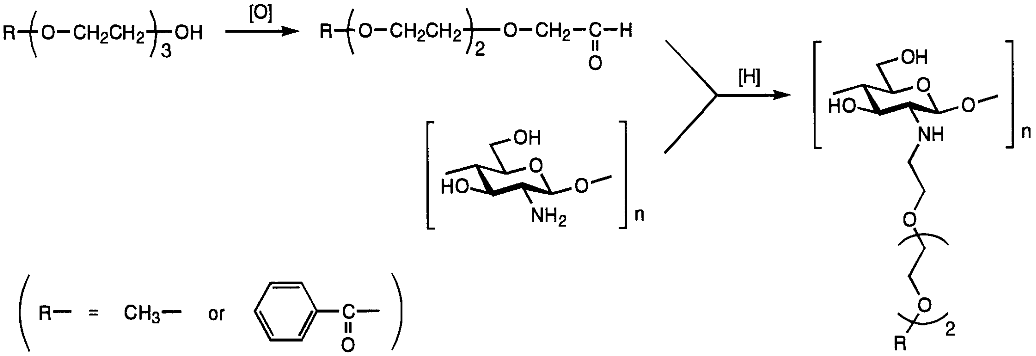
Synthesis of monoaldehydes of oligo(ethylene glycol)s

To introduce oligo(ethylene glycol) branches of the same lengths into chitosan, half-protected oligo(ethylene glycol)s are necessary. While tri(ethylene glycol) monomethyl ether is commercially available, tri- and tetra(ethylene glycol) monobenzoates were prepared by benzoylation of the corresponding oligo(ethylene glycol)s and isolated by repeated column chromatography. The yields of the monobenzoates were about 50% after purification.

Oxidation of the half-protected oligo(ethylene glycol)s was undertaken with various oxidizing agents. The reaction with dimethyl sulfoxide/acetic anhydride proceeded sluggishly at room temperature or even at 60 °C, judging from a weak band due to aldehyde in the IR spectra, and moreover, isolation of the products was difficult. Pyridine/chromium trioxide gave a tar-like substance with no evidence of aldehyde formation, suggesting some decomposition had taken place. Oxidation was then examined with manganese dioxide, which is a mild oxidizing agent suitable for some alcohols with minimal side reactions and easy to be removed after reaction. The reaction was carried out under various conditions, and controlled oxidation to aldehydes was found to be effected with manganese dioxide of about 10 times the weight of the alcohols by heating in toluene. Formation of the aldehydes was confirmed by IR spectroscopy; a moderate to strong band was observed at 1721 cm^{-1}. Isolation of the resulting aldehydes from the starting alcohols was attempted by several methods including chromatography and derivatization, but it was unsuccessful. The content of aldehydes could be determined by the oxime method, and the aldehydes were thus used for the subsequent reductive alkylation in the form of mixtures with the starting alcohols. The yields of aldehydes were 24-40%.

Reductive alkylation of chitosan to form comb-shaped polysaccharides

Reductive alkylation of chitosan with aldehydes and ketones in the presence of an appropriate reducing agent is a convenient method to introduce various groups such as sugars (5), alkyls (6), and carboxyalkyls (7,8). To prepare comb-shaped chitosan derivatives, fully deacetylated chitosan was treated with the monoaldehydes derived from tri(ethylene glycol) under homogeneous conditions in an aqueous acetic acid/methanol solution (Scheme 1). On addition of sodium cyanoborohydride, the solutions became weak gels. The resulting mixtures were dialyzed against deionized water to give clear solutions, which were then concentrated and freeze-dried. The products were obtained as slightly yellow solids.



Scheme 1.

The ds was calculated from the C/N value of elemental analysis. As summarized in Table 1, the ds values were above 1 when excess aldehydes were used. Besides the elemental analysis, IR spectroscopy supported the structure of the products. The introduction of tri(ethylene glycol) branches was evident from the pronounced absorption bands at 2850-2950 and 1000-1200 cm^{-1} as shown in Figure 1. The derivatives having tri(ethylene glycol) benzoate branches showed additional characteristic bands at 1720, 1603, 1277, and 717 cm^{-1} attributable to the benzoyloxy linkage.

Table 1. Preparation of comb-shaped chitosan/tri(ethylene glycol) hybrids

Aldehyde[a]	Equiv[b]	ds[c]	Elemental analysis (%)			Yield[d]
			C	H	N	(%)
TEG-OMe	1	0.83	48.13	8.04	4.75	81
			(48.07	8.20	4.75)[e]	
TEG-OMe	3	1.70	49.72	8.14	3.24	84
			(49.85	8.69	3.25)[f]	
TEG-OMe	5	1.88	49.62	8.22	3.02	77
			(49.70	8.78	3.03)[g]	
TEG-OBz	1.8	1.19	57.62	6.71	3.13	88
			(57.59	6.90	3.13)[h]	
TEG-OBz	3	1.62	57.23	6.82	2.46	53
			(57.29	7.02	2.45)[i]	

[a] Monoaldehydes synthesized from tri(ethylene glycol) monomethyl ether (TEG-OMe) and tri(ethylene glycol) monobenzoate (TEG-OBz).

[b] Mole equivalents of aldehyde to amino groups of chitosan.

[c] Determined from the C/N value of elemental analysis.

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

[e] Calcd for $(C_6H_{11}NO_4)_{0.17}(C_{13}H_{25}NO_7)_{0.83}\cdot 0.7H_2O$.

[f] Calcd for $(C_{13}H_{25}NO_7)_{0.30}(C_{20}H_{39}NO_{10})_{0.70}\cdot 1.2H_2O$.

[g] Calcd for $(C_{13}H_{25}NO_7)_{0.12}(C_{20}H_{39}NO_{10})_{0.88}\cdot 1.5H_2O$.

[h] Calcd for $(C_{19}H_{27}NO_8)_{0.81}(C_{32}H_{43}NO_{12})_{0.19}\cdot 0.3H_2O$.

[i] Calcd for $(C_{19}H_{27}NO_8)_{0.38}(C_{32}H_{43}NO_{12})_{0.62}\cdot 1.3H_2O$.

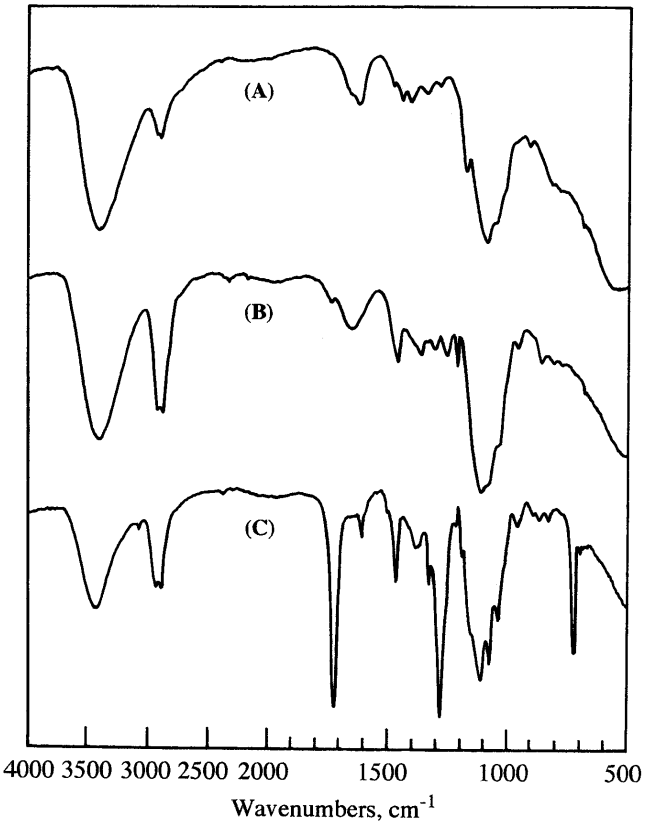


Figure 1. IR spectra of (A) chitosan, (B) chitosan derivative with tri(ethylene glycol) methyl ether branches (ds 0.83), and (C) chitosan derivative with tri(ethylene glycol) benzoate branches (ds 1.19),

Properties of the products

The resulting comb-shaped chitosan/tri(ethylene glycol) hybrids exhibited high affinity for solvents unlike the starting chitin and chitosan. The hybrids were readily soluble in water and ethanol before thorough drying. However, the solubility somewhat decreased on freeze-drying, and they gave highly swollen gels in water and common organic solvents such as alcohols, pyridine, *N*,*N*-dimethylformamide, and dimethyl sulfoxide. The results are summarized in Table 2.

Table 2. Solubility of chitosan and the comb-shaped hybrids

	Solubility[a]				
	Water	Ethanol	Pyridine	DMF	DMSO
Chitosan	–	–	–	–	–
Chitosan-TEG-OMe[b]	+(±)[d]	+(±)[d]	±	±	±
Chitosan-TEG-OBz[c]	+(±)[d]	+(±)[d]	±	±	±

[a] DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; +, soluble; ±, partially soluble or swelled; –, insoluble.

[b] Chitosan having tri(ethylene glycol) methyl ether branches.

[c] Chitosan having tri(ethylene glycol) benzoate branches.

[d] (±), partially soluble or swelled after thorough drying.

Affinity of the products for metal cations was then examined. The pulverized derivatives were treated with aqueous solutions of metal cations, and the adsorption percentages were compared with that of chitosan. As listed in Table 3, the introduction of branches did not interfere much with the adsorption behavior of chitosan, despite the considerable bulkiness of the branches. The capacity for iron(III) decreased as a result of branching, but those for copper(II) and lithium(I) appeared to increase to some extent. These results imply a possibility of favorable participation of the tri(ethylene glycol) chains in the adsorption of metal cations.

Table 3. Adsorption of metal cations by chitosan and the comb-shaped hybrids

	Adsorption (%)[a]				
Adsorbent	Li^+	Na^+	K^+	Fe^{3+}	Cu^{2+}
Chitosan	4.3	7.0	9.3	67.0	34.7
Chitosan-TEG-OMe[b]	7.8	8.0	8.2	28.8	42.8
Chitosan-TEG-OBz[c]	6.2	5.4	7.6	45.9	43.1

[a] Adsorbent, 10 mg; metal ion solution, 5 mmol/L, 10 mL; temp, 25 °C; time, 24 h.

[b] Chitosan having tri(ethylene glycol) methyl ether branches with ds 0.83.

[c] Chitosan having tri(ethylene glycol) benzoate branches with ds 1.19.

Conclusion

Comb-shaped chitosan derivatives have been prepared by reductive alkylation with monoaldehydes of tri(ethylene glycol) that were synthesized by controlled oxidation of the corresponding monoalcohols. The introduction of such branches markedly increased affinity for both water and organic solvents without sacrificing the attractive characteristics of chitosan such as metal cation adsorption capacity. The results suggest a feasibility of preparing a new type of chitosan derivatives with finely regulated structures leading to development of advanced functions.

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