Synthesis of a Chitosan−Dendrimer Hybrid and Its Biodegradation†

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Chitosan−dendrimer hybrids having various functional groups such as carboxyl, ester, and poly(ethylene glycol) groups were prepared successfully using dendrimer acetal by reductive *N*-alkylation. The synthetic procedure could be accomplished by one-step reaction without organic solvent. The degree of substitution of dendrimer was 0.13−0.18 evaluated by ^{1}H NMR. A perfectly or partially water-soluble chitosan−dendrimer hybrid could be obtained. By standard activated sludge, good biodegradation was observed in these hybrids.

Introduction

Chitosan, a polysaccharide formed primarily of repeating D-glucosamine units, shows some biological activities in gene delivary,[2] antibacterial activity,[3] and wound healing activity.[4] Chitosan is also nontoxic and biodegradable in animal body.[5] Moreover a chitosan derivative, *N*-carboxybutylchitosan, is applied for wound healing dressings and tissue repair promoters owing to its significant biological activities.[6] On the other hand, dendrimers prepared by divergent or convergent methods are monodispersed macromolecules. They represent a chemically well-defined structure.[7] By molecular design, dendrimers offer numerous possibilities for medical applications, host−guest chemistry, dendritic catalysts, etc. owing to their multifunctional properties.[8,9] Therefore, the dendrimer is one of the attractive molecules to modify with chitosan. Moreover, the chitosan−dendrimer hybrid (CDH) is a novel type molecule like "tree type molecule" which can lead a variety of functional molecules at the surface of dendrimer such as sugar, peptide, lipid, drug, and so on.

We have reported so far about the synthesis on several kinds of CDHs, which bound sialic acid at the terminal group of dendron.[10] Our previous works, however, did not include any biological property. Herein, we report the synthesis of CDHs bound ester, carboxyl, and poly(ethylene glycol) (PEG) groups at the terminal of dendron, and their biodegradable property.

Experimental Section

Materials. Chitosan (SK-10: $NH_2 = 0.85$) was supplied from Koyo chemical Co., Japan. Aminoacetaldehyde dimethyl acetal, aminoacetaldehyde diethyl acetal, methyl acrylate, and PEG acrylate (DP of PEG = 7) were purchased from Aldrich Co., Ltd. and used without further purification.

General Methods. ^{1}H NMR spectra were recorded on JEOL A-500 NMR spectrometer. The molecular weight was determined by means of GPC using pullulan as standards (column, Tosoh TSK Gel G4000pwxl and G3000pwxl; eluent, 0.5 M AcOH-0.5 M AcONa buffer; temp, 40 °C; flow rate, 1.0 mL/min; detection, RI). Biodegradation of chitosan and its derivatives with standard activated sludge was evaluated by BOD tester 100F (TAITEC Co., Japan) according to the previous report.[11]

The biodegradation (%) after 27 days was calculated as the following equation:

$$\% = [\text{experimentally consumed } O_2 \text{ (mL)}/\text{theoretical } O_2 \text{ (mL)}] \times 100$$

The solubility of the product in water was evaluated from the visual observation method in which 10 mg of product was suspended in water (2 mL) for 1 day. Amorphous chitosan was prepared as follows. Chitosan (1.0 g) was dissolved in 0.2 M AcOH (50 mL). To the solution was added 1 M NaOH (12 mL). The mixture was dialyzed for 2 days and lyophilized to obtain amorphous chitosan (0.96 g).

Preparation of Dendrimer Acetals. Preparation of dendrimer acetals (**1a**−**5a**) was reported previously.[9c] The preparation of PEG terminal dendrimer acetals (**1b**, **3b**, and **5b** in Scheme 1) is as follows. Aminoacetaldehyde diethyl acetal and PEG acrylate (1.1 equiv/NH_2) were dissolved in MeOH (50 mL). The solution was stirred at 50 °C for 4 days. The solution was concentrated to dryness to give acetal **1b** without further purification. Acetals **3b** and **5b** were prepared from dendrimer **2** and **4** by the same procedure as above. Acetals (**1c** and **1d** in Scheme 2) were also prepared in EtOH as solvent using ethyl acrylate by the above procedure. ^{1}H NMR spectral data of **1a**−**5a** have been reported previously.[9c]

Data for **1b**: ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 6.0 H, CH_3 of diethyl acetal), 2.46 (t, 4 H, CH_2 (b)), 2.61 (d, 2 H, CH_2

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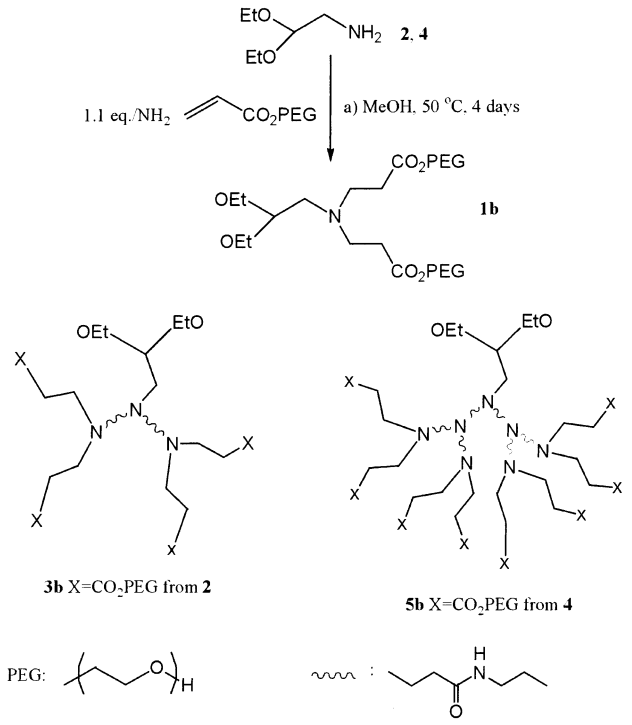
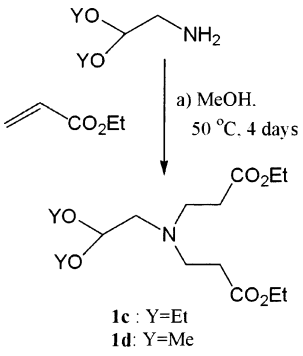
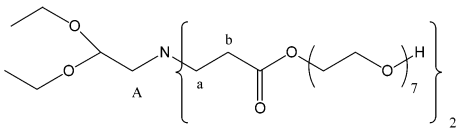
Scheme 1



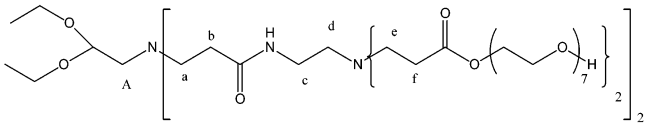
Scheme 2



(A)), 2.87 (t, 4 H, CH_2 (a)), 3.5−3.8 (m, 60 H, CH_2 of PEG and CH_2 of diethyl acetal), 4.50 (t, 1 H, CH of acetal).

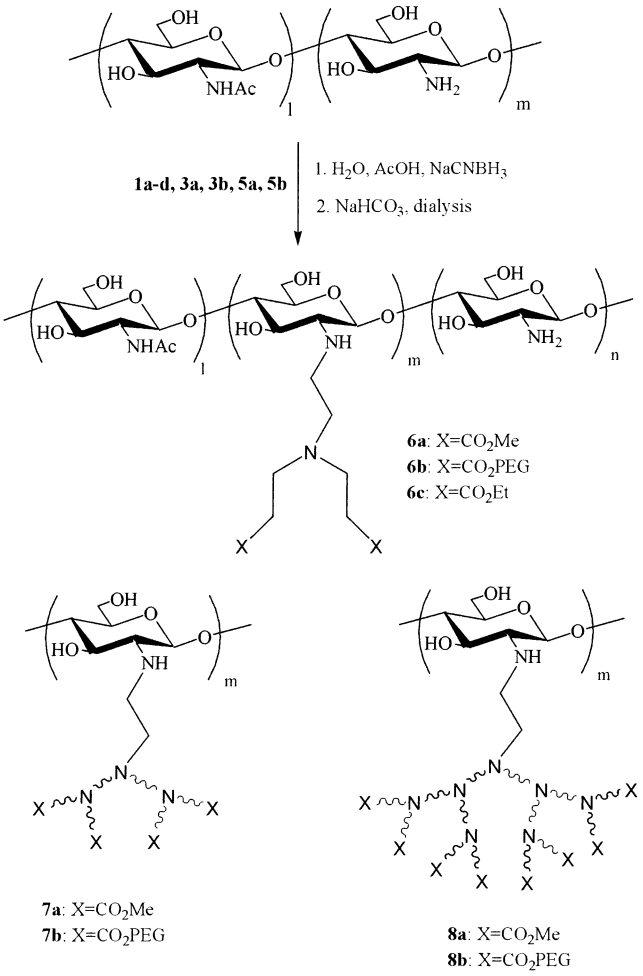


Data for **3b**: ^{1}H NMR ($CDCl_3$) δ 1.19 (t, 6.0 H, CH_3 of diethyl acetal), 2.37−2.54 (m, CH_2 (b, f)), 2.64 (d, 2 H, CH_2 (A)), 2.70−2.90 (m, CH_2 (a, c, d, e)), 3.3−3.8 (m, CH_2 of PEG and CH_2 of diethyl acetal), 4.57 (t, 1 H, CH of acetal), 7.29 (br, 2H, NHCO).

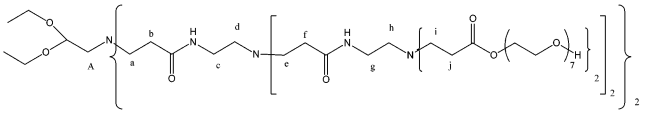


Data for **5b**: ^{1}H NMR ($CDCl_3$) δ 1.19 (t, 6.0 H, CH_3 of diethyl acetal), 2.37−2.64 (m, CH_2 (b, f, j, A)), 2.70−2.90 (m, CH_2 (a, c, d, e, g, h, i)), 3.3−3.8 (m, CH_2 of PEG and

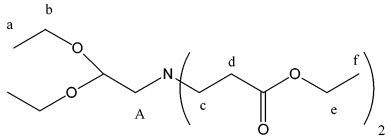
Scheme 3



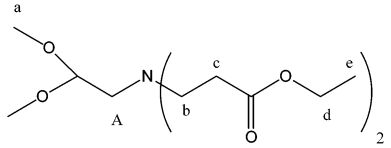
CH_2 of diethyl acetal), 4.57 (t, 1 H, CH of acetal), 7.0−7.8 (m, NHCO).



Data for **1c**: ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 6.0 H, CH_3 (a)), 1.25 (t, 6.0 H, CH_3 (f)), 2.45 (t, 4 H, CH_2 (d)), 2.61 (d, 2 H, CH_2 (A)), 2.87 (t, 4 H, CH_2 (c)), 3.51−3.68 (m, 4 H, CH_2 (b)), 4.11 (dd, CH_2 (e)), 4.50 (t, 1 H, CH of acetal).



Data for **1d**: ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 6.0 H, CH_3 (e)), 2.45 (t, 4 H, CH_2 (c)), 2.61 (d, 2 H, CH_2 (A)), 2.87 (t, 4 H, CH_2 (b)), 3.35 (s, 6 H, CH_3 (a)), 4.11 (dd, CH_2 (d)), 4.39 (t, 1 H, CH of acetal).



Scheme 4

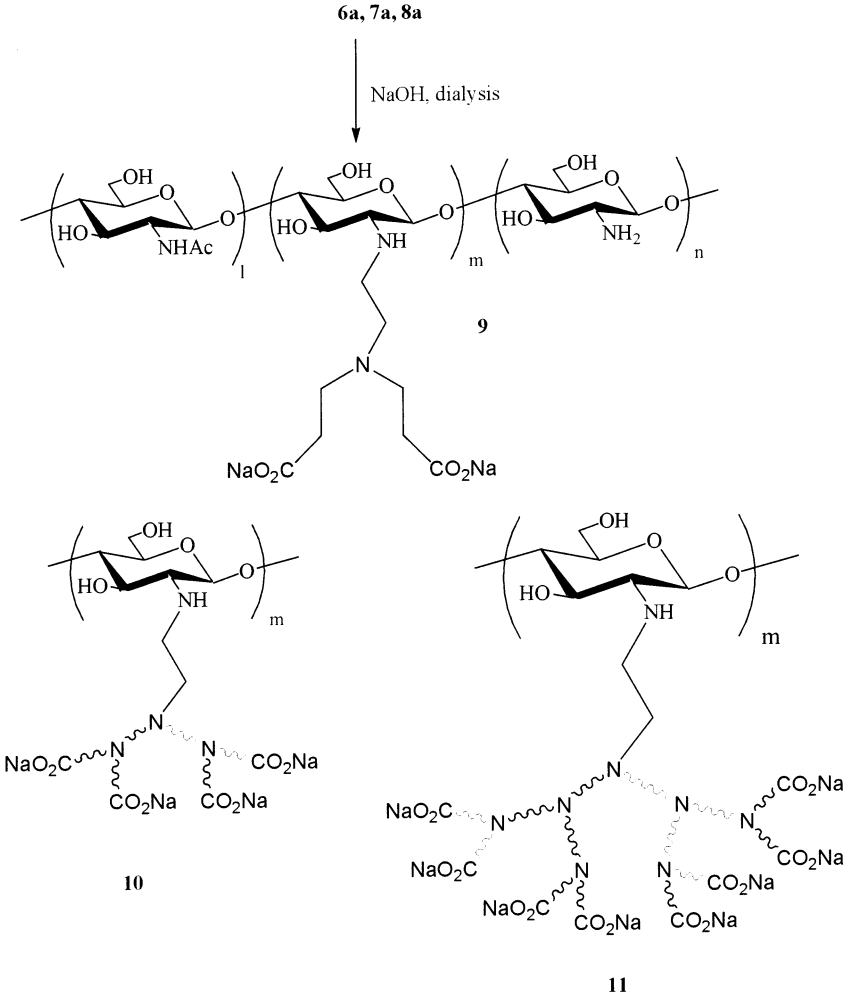
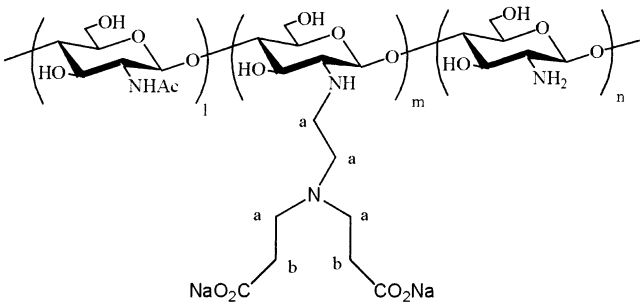


Table 1. Chemical Shifts of Compounds in ^{1}H NMR

	chemical shift[a]/ppm					
compd	NHAc	b	H-2	H-1	others	CH_3
6a	2.06 (0.45 H)	3.01 (0.80 H)	3.2−3.3 (0.85 H)	4.9−5.2 (0.85 H)	3.6−4.1	
6b	2.06 (0.45 H)	3.01 (0.72 H)	3.31 (0.85 H)	4.9−5.2 (0.85 H)	3.6−4.1	
6c	2.06 (0.45 H)	3.01 (0.60 H)	3.2−3.3 (0.85 H)	4.9−5.2 (0.85 H)	3.6−4.1	1.27 (0.75H)
7a	2.06 (0.45 H)	3.01 (1.20 H)	3.2−3.3 (0.85 H)	4.9-5.2 (0.85 H)	3.6−4.1	
7b	2.06 (0.45 H)	3.01 (1.28 H)	3.31 (0.85 H)	4.9−5.2 (0.85 H)	3.6−4.1	
8a	2.06 (0.45 H)	3.01 (2.08 H)	3.2−3.3 (0.85 H)	4.9−5.2 (0.85 H)	3.6−4.1	
8b	2.06 (0.45 H)	3.01 (2.24 H)	3.31 (0.85 H)	4.9−5.2 (0.85 H)	3.6−4.1	
9	2.07 (0.45 H)	3.01 (0.80 H)	3.0-3.2(0.85 H)	4.9−5.2 (0.85 H)	3.6−4.1	
10	2.07 (0.45 H)	2.8−2.9 (1.2 H)	3.0−3.2(0.85 H)	4.9−5.2 (0.85 H)	3.6−4.1	
11	2.07 (0.45 H)	2.8−2.9 (2.1 H)	3.0−3.2(0.85 H)	4.9−5.2 (0.85 H)	3.6−4.1	

[a] H-2, H-2 of GlcN and N-alkylated GlcN residue; H-1, H-1 of N-alkylated GlcN residue; CH_3, CH_3 of CO_2Et; b, methylene proton, which shows the following structure



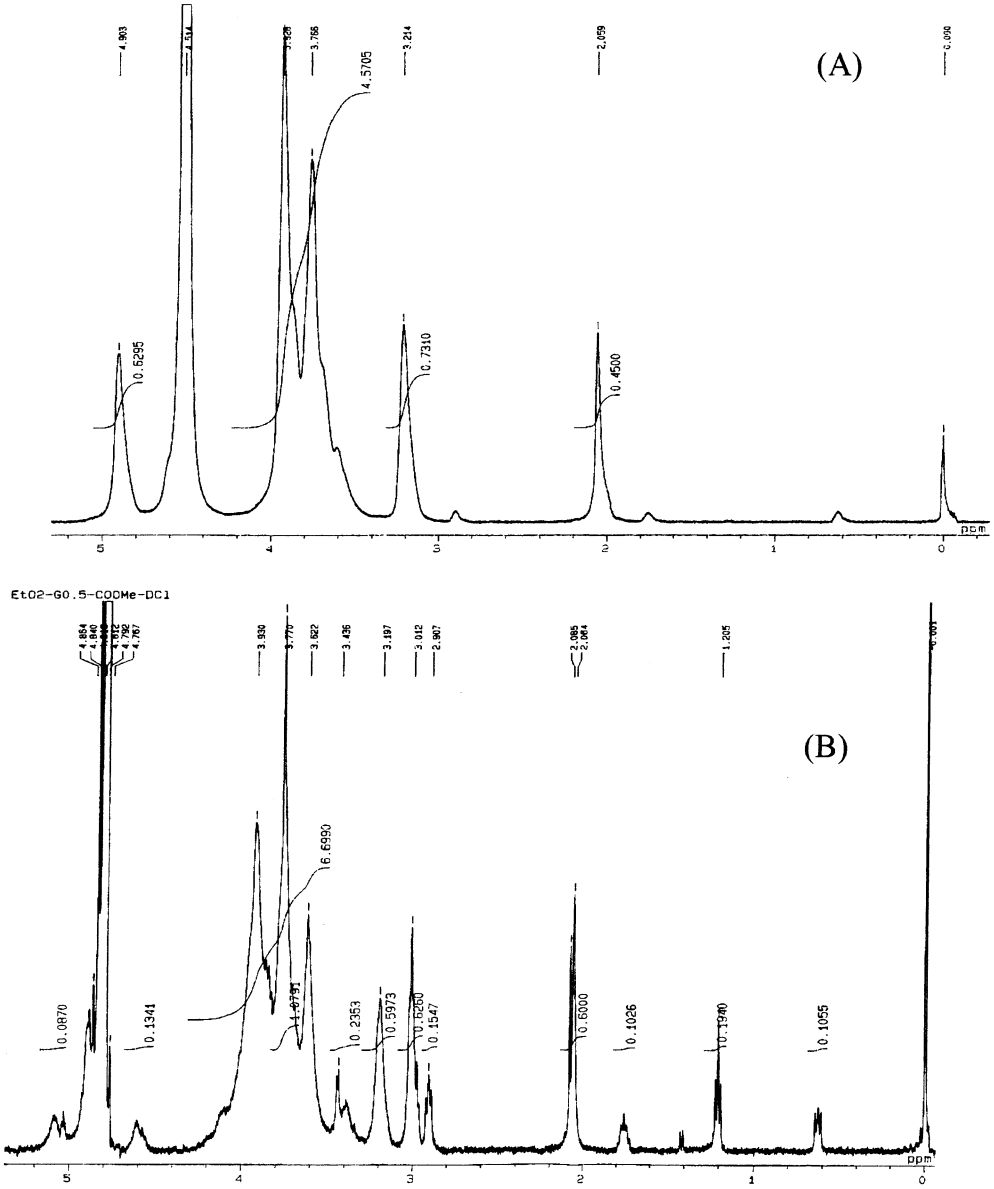


Figure 1. ^{1}H NMR spectra of (A) chitosan and (B) CDH (**6a**).

Synthesis of Chitosan−Dendrimer Hybrid (CDH). The typical procedure is as follows. Chitosan (0.4 g) and acetal (2 equiv/NH_2) were dissolved in H_2O (20 mL) containing AcOH (4 mL), and then $NaCNBH_3$ (4 equiv/NH_2) was added. The mixture was stirred at room temp. After 1 day, $NaHCO_3$ was added to the mixture to adjust the pH at 8. The mixture was dialyzed for 2 days, lyophilized, washed with EtOH to remove remained acetal, and dried to obtain CDH (Scheme 3). The saponification of CDH is as follows (Scheme 4). CDHs (**6a**, **7a**, **8a**) were suspended in 0.1 M NaOH (20 mL) at room temperature for 1 h. The mixture was dialyzed for 2 days and lyophilized to obtain carboxyl group terminated CDHs (**9**, **10**, **11**).

Structural Analysis. The DS of CDH per one repeating glycopyranose unit was determined by ^{1}H NMR in 0.5 M DCl/D_2O from the peak area at δ 2.91 (−C**H**$_2$−CO_2R) against 2.05 (NHCOC**H**$_3$ of chitosan: 0.45 H). The DS of ester groups was also determined by ^{1}H NMR from the peak area at δ 1.26 (CO_2CH_2C**H**$_3$) or 3.6 (COC**H**$_3$) against 2.05 (NHCOC**H**$_3$ of chitosan: 0.45 H). The chemical shifts of compounds **6**−**10** are listed in Table 1.

Results and Discussion

Synthesis of CDH. In our previous study, the dendrimer acetals were transformed into the corresponding aldehydes with CF_3CO_2H and 2 M HCl, and then these aldehydes reacted with chitosan in aq. AcOH and MeOH mixed solvents.[10c] This procedure, however, was not appropriate because of the two step reaction and using MeOH as an organic solvent. Recently, a more beneficial reaction has been reported using 2,2-dimethoxypropane (dimethyl acetal) in aq. AcOH as a solvent.[12] According to this, chemical modification of chitosan with dendrimer acetals can be performed by one step without any organic solvent like MeOH. The reaction of chitosan with various dendrimer acetals in aq. AcOH is shown in Scheme 3. Although these acetals (**1**, **3**, **5**) were insoluble in water, they dissolved in aq. AcOH,

Table 2. Synthesis of Chitosan−Dendrimer Hybrid[a]

starting material	terminal structure	product	yield %	DS	DS of CO_2R[b]	solubility in H_2O[c]	generation
1a	$(CO_2Me)_2$	**6a**	95	0.16	0.26	yes	1
1b	$(CO_2PEG)_2$	**6b**	90	0.18		yes	1
1c	$(CO_2Et)_2$	**6c**	88	0.14	0.24	yes	1
1d	$(CO_2Et)_2$	**6c**	90	0.15	0.26	yes	1
3a	$(CO_2Me)_4$	**7a**	92	0.15	0.48	part	2
3b	$(CO_2PEG)_4$	**7b**	96	0.16		part	2
5a	$(CO_2Me)_8$	**8a**	89	0.13	0.80	part	3
5b	$(CO_2PEG)_8$	**8b**	95	0.14		part	3
6a	$(CO_2Na)_2$	**9**	90	0.16	0	yes	1
7a	$(CO_2Na)_4$	**10**	96	0.15	0	yes	2
8a	$(CO_2Na)_8$	**11**	92	0.13	0	yes	3

[a] Reaction condition: chitosan, 0.4 g; H_2O, 20 mL; AcOH, 4 mL, acetal, 2 equiv/NH_2; $NaCNBH_3$, 4 equiv/NH_2; room temp.; 1 day. [b] CO_2R, DS of ester groups per one repeating unit. [c] part: partially soluble in H_2O.

Table 3. Formular Weight (FW) and Reactivity of Dendrimer Acetals

acetal	FW[a]	reactivity[b]/%
1a	305	9.4
1b	883	10.6
1c	333	8.3
1d	305	8.9
3a	706	8.9
3b	1861	9.4
5a	1506	7.7
5b	3818	8.3

[a] FW was calculated from the chemical structure of acetal. [b] Reactivity/% = (DS/equiv of acetal added × 0.85) = (DS/2 × 0.85) × 100. 0.85 means mol fraction of chitosan.

Table 4. Molecular Weight of Chitosan and CDHs

compd	terminal structure	DS	Mn	Mw	Mw/Mn
amorphous chitosan		0	40 200	100 000	2.4
6a	$(CO_2ME)_2$	0.16	36 000	98 000	2.7
6b	$(CO_2PEG)_2$	0.18	46 000	163 000	3.5
7a	$(CO_2Me)_4$	0.15	46 000	98 000	2.1
7b	$(CO_2PEG)_4$	0.16	54 000	210 000	3.9
8a	$(CO_2Me)_8$	0.13	53 000	120 000	2.3
8b	$(CO_2PEG)_8$	0.14	59 000	234 000	4.0

Table 5. Biodegradation of Amorphous Chitosan and CDHs

compd	terminal structure	DS	solubility in H_2O	biodegradation after 27 days
chitosan		0	no	33.0
6a	$(CO_2ME)_2$	0.16	yes	12.8
6b	$(CO_2PEG)_2$	0.18	yes	11.2
9	$(CO_2Na)_2$	0.16	yes	6.0
7a	$(CO_2Me)_4$	0.15	part[a]	7.2
7b	$(CO_2PEG)_4$	0.16	part	9.1
10	$(CO_2Na)_4$	0.15	yes	4.6
8a	$(CO_2Me)_8$	0.13	part	9.0
8b	$(CO_2PEG)_8$	0.14	part	2.8
11	$(CO_2Na)_8$	0.13	yes	4.8

[a] part: partially soluble in H_2O.

because of their salt formation at *tert*-amino groups. Figure 1 shows the ^{1}H NMR spectra of chitosan (A) and CDH (**6a**: B). A typical signal corresponding to $-CH_{2b}-CO_2R$ was observed at $\delta = 3.01$ ppm. The DS of CDH could be estimated by the signal at $\delta = 3.01$ ppm against $NHCOCH_3$ at $\delta = 2.06$ ppm. The signals at $\delta = 3.4$ and 5.1 ppm were assigned to H-2 and H-1 protons of the *N*-alkyl group substituted glucosamine residue, which indicates the dendrimer was surely bound to the chitosan backbone. The minor signals at $\delta = 1.20$ and 2.09 ppm were due to the contaminants of EtOH (0.19 H) and AcONa (0.15 H).

The results on the preparation of CDH are summarized in Table 2. When 2 equiv of dendrimer acetal was used, the DS of CDH showed 0.13−0.20. The reactivity of acetals to chitosan in 2.8 M AcOH was 7.7−10.6%, which was about half degree of reactivity by previous procedure (21−25%).[9c] The reactivity of dimethyl acetal (**1d**: 8.9%) was almost the same as that of diethyl acetal (**1c**: 8.3%). Although the reactivity of acetal used in this study was lower than that of previous aldehyde,[9c] the moderate reactivity of dendrimer acetals to chitosan could be achieved in this medium and CDHs were obtained in excellent yields (88−96%). From the ^{1}H NMR analysis of CDH and Table 2, a part of methyl ester was removed. The water solubility was much improved by the modification of the dendrimer in comparison with original chitosan, which did not dissolve in water. The CDHs with low generation (**6a**−**d**) and those having carboxyl groups (**9**−**11**) dissolved perfectly in water. Other CDHs showed partial dissolution in water. The transformation of ester to carboxyl group was effective on the water-solubility of CDH independent of the generation of CDH. Partial dissolution in CDHs of high generation (**7** and **8**) would be caused by steric hindrance by a large dendrimer moiety (dendron).

Table 3 shows the formular weight (FW) and the reactivity of dendrimer acetals. The reactivity of acetal was almost independent of the terminal structure and FW of acetal. In our previous study,[9c] the reactivity of acetal was remarkably decreased over FW 6300 owing to its steric hindrance. The reactivity of acetals was not affected, because the FWs of acetals used in this study were below 3800. Table 4 shows the molecular weight (MW) of chitosan and CDHs. The MWs of CDHs (**6a**, **7a**, and **8a**) were similar to that of original chitosan. In contrast, the MW of CDHs (**6b**, **7b**, and **8b**) possessing a PEG group remarkably increased, especially for Mw. This suggests that the high FW of the dendrimer acetal was surely bound to chitosan and resulted in MW increasing.

Biodegradation of Amorphous Chitosan and CDHs. Because all CDHs used for the biodegradation test were of the amorphous form, original chitosan was also tested as an

amorphous state. The biodegradations of amorphous chitosan (AC) and various CDHs by standard activated sludge are summarized in Table 5. CDHs having a carboxyl group (**9**−**11**) were at a disadvantage for the biodegradation despite their water-solubility. The CDH (**8b**) having the largest dendritic size showed lowest biodegradation. Other CDHs (**6**, **7**, and **8a**) maintained good biodegradation (7.2−12.8%), although these were lower than that of water-insoluble AC (33%). Therefore, the biodegradation is independent of the water-solubility, but much depends on the chemical structure. The decrease of biodegradation would be caused by the fact that the degradation by enzyme was inhibited by steric hindrance of dendrons.

Conclusion

In this study, CDHs having functional groups (carboxyl, ester, and PEG) and various generations were successfully prepared. The synthetic procedure could be improved to be a one-step reaction without organic solvent. The solubility in water was also improved to modify chitosan by these dendrimers. Good biodegradation was observed in CDHs (**6a**, **6b**, **7a**, **7b**, and **8a**). These CDHs would be useful for further application as biodegradable, biomedical, or supermolecular polysaccharides.

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