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Novel synthesis of a water-soluble cyclodextrin-polymer having a chitosan skeleton

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Formation of Schiff's base between 2-*O*-(formylmethyl)-β-cyclodextrin and chitosan with an average molecular weight of 40 000 in acetate buffer at pH 4.4, followed by reduction with sodium cyanoborohydride produced a β-cyclodextrin-linked chitosan in a one-pot reaction. The product, which had a degree of substitution of 37%, was soluble in water at neutral and alkaline conditions. UV-visible and circular dichroism spectroscopic examinations revealed that the product had the ability to form a host-guest complex with *p*-nitrophenolate. © 1998 Elsevier Science Ltd. All rights reserved.

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Introduction

Chitosan, 2-amino-2-deoxy-β(1,4)-glucan, is a multifunctional polymer readily prepared by de-*N*-acetylation of natural mucopolysaccharide chitin. Chitosan has been widely used as an antifungal substance[1], metal-ion adsorbant[2,3], and ion exchanger[4], etc. The polycationic property of chitosan that is soluble in water under acidic conditions but insoluble under neutral or basic conditions provides casting thin films or microporous beads[5]. Among numerous studies on chemical modification of chitosan[6], we have recently succeeded in creating a chitin-based supramolecular species through condensation of carboxymethylated β-cyclodextrin (β-CD) and a chitosan oligomer (M.w. 7300) via formation of an amide bond[7]. This CD-linked chitosan oligomer was found to possess the ability to form host-guest complexes with a fluorescent dye [7]. However, application of this methodology to chitosan with a higher molecular weight failed, because the solubility is extremely low in water under the neutral or alkaline conditions that are necessary for the condensation reaction. Surveying new coupling reactions between CD and polymeric chitosan, we focused on Hanessian's report [8] concerning the preparation of CD derivatives with an aldehyde function because reductive amination has been one of the major reactions applicable to the modification of chitosan [9]. Here, we report a convenient method for introducing a β-CD residue into chitosan with a high molecular weight using reductive amination as the key reaction.

Experimental

Materials. Chitosan was prepared by treatment of Flonac C (Kyowa Technos Co. Ltd, Chiba, Japan), partially de-*N*-acetylated chitin with an average molecular weight 40 000, with 50% (wt/v) aqueous NaOH for 2 h at 120°C three times[10]. 2-*O*(Formylmethyl)-β-cyclodextrin **2** was prepared by the slightly modified procedure of Hanessian[8] through allylation of β-CD, separation with Diaion HP-20 to separate the mono 2-*O*-allyl-β-cyclodextrin **1**, and ozonolysis of the allyl group in **1**. The reaction was monitored by TLC on a precoated Silica gel 60 F_{254} (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany) using *n*-BuOH–EtOH–H_2O (6:4:3, v/v/v) as the eluant.

Preparation of β-CD-chitosan. To a solution of chitosan (200 mg) in 0.2 M acetate buffer at pH 4.4 (100 ml) was added a solution (50 ml) of **2** that was prepared form **1** (1 g) was added by portions. The mixture was stirred for 1 h at room temperature. Sodium cyanoborohydride (260 mg) was added to the resulting solution. The mixture was stirred for 4 days at room temperature, neutralized with 5% ammonia water, subjected to ultrafiltration through a membrane (molecular weight cutoff, 10 000), and lyophilized to give the β-CD-linked chitosan **4** (0.86 g, 57% yield) with degree of substitution of 37%; $[\alpha]_D$ 107°(*c* 0.27, 2% aq. AcOH); Anal. Found: C, 44.22; H, 6.36; N, 2.35%. Calculated for $[C_6H_{11}NO_4(C_{44}H_{72}O_{35}\cdot 2H_2O)_{0.37}]_n$: C, 44.30; H, 6.53; N, 2.32%.

Spectroscopic analysis of the CD-linked chitosan. ^1H and ^{13}C n.m.r. spectra were recorded at 300.13 and 75.47 Hz with a Bruker ASX-300 spectrometer in deuterium oxide. Inclusion ability was estimated using a Hitachi U-3200 spectrometer to record the UV-visible spectrum of a solution of 4 in 0.05 M phosphate buffer (pH 8.7) containing *p*-nitrophenol (PNP; 7.9 mg l^{-1}) as the guest compound. Circular dichroism spectrum of the CD-chitosan-PNP complex was recorded with a JASCO J-720 spectropolarimeter in the same solvent system.

Results and discussion

β-CD was allylated according to Hanessian's method[8] and purified by reverse phase chromatography on Dialon HP-20 to give mono 2-*O*-allyl-β-CD **1**. Oxidation of the

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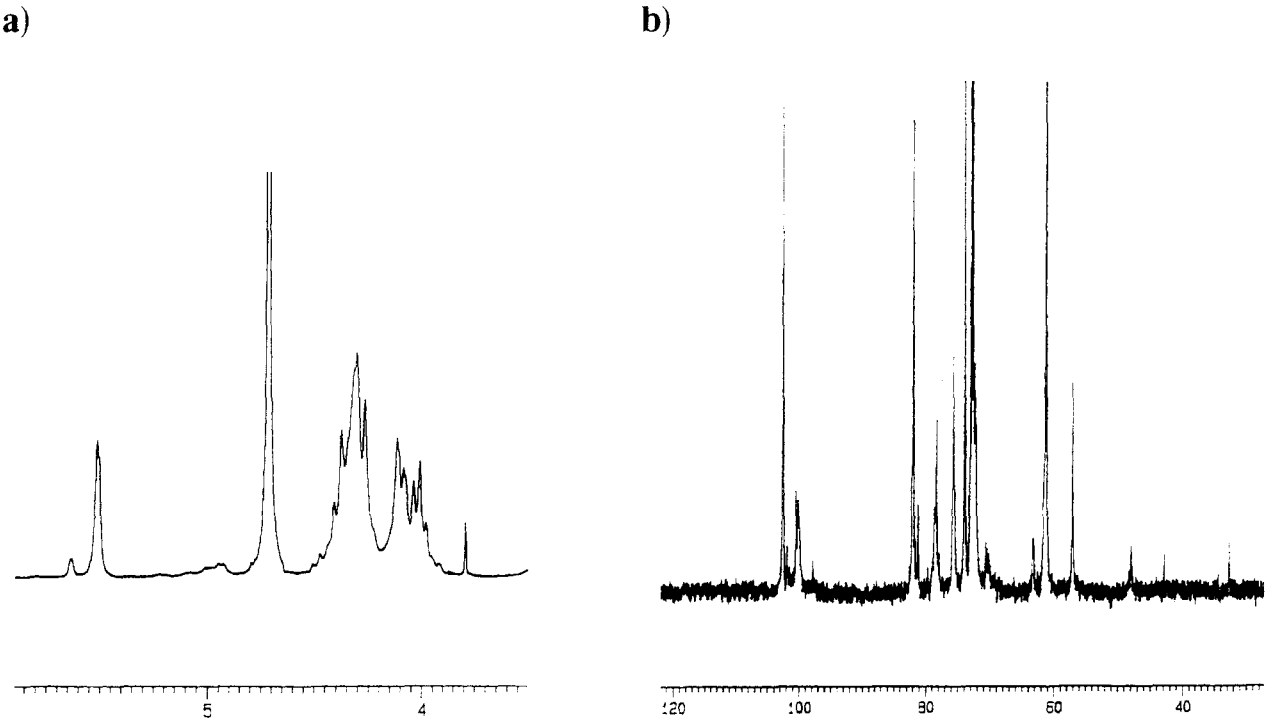
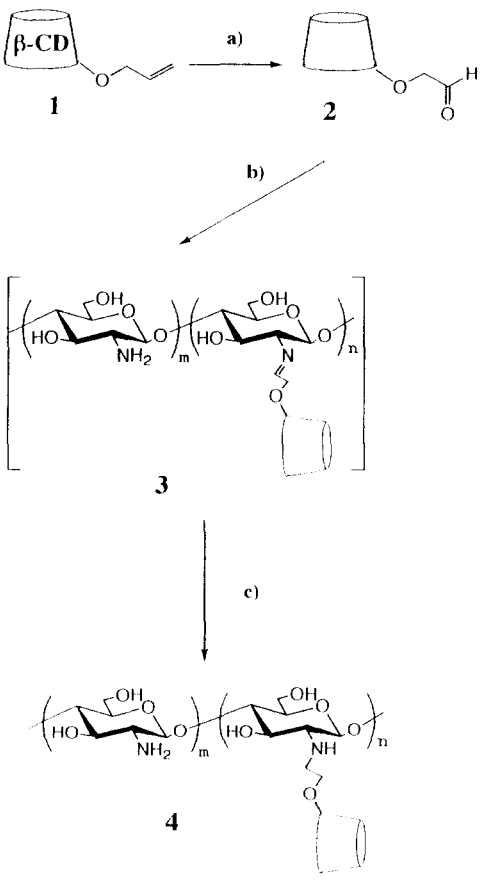


Figure 1 (a) ^{1}H-n.m.r. spectrum at 300.13 Hz and (b) ^{13}C-n.m.r. spectrum at 75.47 Hz of β-CD-linked chitosan with a D.S. of 37% recorded in deuterium oxide at 60°C.

C–C double bond in **1** with ozone in aqueous methanol and subsequent reductive work-up using dimethyl sulfide afforded an aldehyde, 2-*O*-(formylmethylated)-β-CD **2**. Because the solubility of chitosan is higher in the acidic media and the formation of Schiff's base is accelerated at lower pH region, the reductive coupling between **2** and high-molecular weight chitosan was to be conducted in acetate buffer at pH 4.4. Thus, **2** and chitosan in the ratio of 5:1 (wt/wt) were stirred at room temperature together to form a Schiff's base **3**, which was directly subjected to reduction with 5 molar equivalents of sodium cyanoborohydride at room temperature to give the corresponding secondary amine. The desired β-CD-linked chitosan **4** was isolated by neutralization of the reaction mixture with ammonia water followed by ultrafiltration through a membrane with molecular cut-off of 10000 and lyophilization.

The structure of **4** was characterized by ^{1}H- and ^{13}C-n.m.r. spectroscopy for solution in D_2O at 60°C, as shown in *Figure 1*. In the ^{13}C-n.m.r. spectrum of CD-chitosan **4**, the signals for the anomeric carbons of the cyclodextrin moiety and chitosan were observed at δ 103–104 and 100.6 ppm, respectively. In addition, the two methylene carbons linking the cyclodextrin and glucosamine residues appeared at δ 32.7 and 58.0 ppm. On the other hand, the ^{1}H-n.m.r. spectrum of **4** showed the anomeric protons of α-D-glucopyranosyl residues in the β-CD moiety at δ 5.5–5.6 ppm and that of 2-amino-2-deoxy-β-D-glucopyranosyl residues in the chitosan skeleton at δ 4.9–5.1 ppm. From the area ratio of the signals of these anomeric protons, the degree of substitution (D.S.) of he product **4** was estimated to be 37%, which was in agreement with the value calculated from elemental analysis data (*Scheme 1*).

Furthermore, similar coupling reaction using **2** and chitosan in the ratio of 1:1 (wt/wt) gave a lower substituted product of **4**, of D.S. was estimated to be 7% by ^{1}H-n.m.r.



Scheme 1 Reagent and conditions: (a) O_3 in 20% aqueous MeOH, −15°C, 4 h, and then Me_2S, room temperature, overnight; (b) chitosan (average molecular weight of 40000), acetate buffer (pH 4.4), room temperature, 1 h; (c) $NaBH_3CN$, acetate buffer (pH 4.4), room temperature 4 d.

spectroscopy in deuterium oxide-acetic acid-d_4. The novel CD-linked chitosan was soluble in water only under acidic conditions, as was the case with the original chitosan. By contrast, **4** with a D.S. of 37% was found to be dissolved in distilled water or even in alkaline solvents such as aqueous ammonia and aqueous sodium hydroxide as well as under acidic conditions.

The inclusion ability of the β-CD-linked chitosan **4** with a D.S. of 37% was examined in terms of UV-visible spectroscopy using *p*-nitrophenolate (PNP) as a guest compound in a phosphate buffer at pH 8.7. Similar to the parent β-CD, a bathochromic shift was observed in the UV-visible spectrum with increasing concentration of the host molecule **4**. Isosbestic points were observed at 334, 400 and 455 nm. Use of the Benesi–Hildebrand equation[11], calculated on the basis of the β-cyclodextrin residues in **4**, showed that the dissociation constant of the host-guest complex with **4** and PNP was 1.49×10^{-3} M, which was comparable to that of the parent β-CD ($K_D = 1.3 \times 10^{-3}$ at pH 10)[12]. Furthermore, the inclusion ability of the β-CD-linked chitosan **4** towards PNP was also revealed by the large induced circular dichroism effect observed at 400 nm. This is probably due the achiral dye is trapped in chiral surroundings consisted of the β-CD-polymer.

In conclusion, the reductive amination of a β-CD derivative bearing an aldehyde function with polymeric chitosan yielded a novel CD-linked polymer that possessed an inclusion ability with *p*-nitrophenolate. This novel polymeric host compound having a carbohydrate skeleton would be useful in cosmetic or pharmaceutical industries in addition to analytical chemistry[13]. Further studies along this line are now in progress.

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