Preparation of Spherical Nano Particles Using Novel Oligosaccharide Pendant Polymers and their Characterization

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Oligosaccharide-pendant vinyl monomers were synthesized without the use of any protecting group for a oligosaccharide component. A high yield of 1-amino- β -saccharide was obtained by reductive amination of the oligosaccharide. 2-isocyanatoethyl methacrylate was used as a polymerizable moiety because no by products, especially acids or salts, were not created. The chemical structures were confirmed by ¹H-NMR and IR spectroscopies. The obtained macromonomers were polymerized in an aqueous solution by redox-initiators using ammonium persulfate and *N*,*N*,*N*'.*N'*-tetraethylethylenediamine. The molecular weight of the polymer was evaluated by size-exclusion chromatography. These results indicate that the polymer forms huge aggregates in an aqueous solution caused by hydrogen bonding. We synthesized glucose, cellobiose, and cellotriose derivatives by the same synthetic procedure and obtained polymerized oligosaccharide derivatives in order to compare aqueous nanodispersions of these polymers was evaluated by size-exclusion chromatography (SEC). The molecular weights of the polymers were extremely huge ($M_w > 10^7$) in an aqueous solution as a result of the SEC determination. The elution time also indicates that the polymer sizes were extremely huge in water. The TEM and dynamic light scattering (DLS) observation showed formation of the nano-size spherical particles.

Key words: oligosaccharide pendant monomer, self aggregation, nano particles, TEM, DLS

1. INTRODUCTION

Recently, nano-sized spherical particles consisting of various water-soluble polymers have been reported [1-6] as functional materials possessing potential applications, including drug delivery [7], enzyme encapsulation, [8] and nano particle catalysis [9, 10], due to structural characteristics such as controllable permeability into the inner cavity, low density, large specific area, and nanosizing. We believe that utilization of natural resources is also essential to the development of novel materials. We therefore turned our attention to cellulose as a natural resource. Cellulose is one of the most abundant biomacromolecules on the earth of renewable organic raw materials [11-14]. However, cellulose is quite rigid due to derived from inter- and intramolecular hydrogen bondings. Therefore, cellulose has poor workability due to its thermal stability, the poor controllability of its chemical reactions, and its low solubility in water and common organic solvents [15]. To improve the these properties, we have developed constitutional units of cellulose, such as glucose, cellobiose and cellotriose, pendant vinyl monomers, and converted their monomers into corresponding polymers as a new type of watersoluble polymer [16]. In the present study, we have confirmed the chemical structure of synthetic products and demonstrated that huge aggregates formed from cello-oligosaccharide pendant polymers are spherical nano particles using size-exclusion chromatography, transmission electron microscopy and dynamic light scattering observations.

2. EXPERIMENTAL

2.1 Chemicals

D(+)-Glucose was obtained from Wako Pure Chemical Industries, Ltd. (Japan). D(+)-Cellobiose was obtained from Tokyo Chemical Industry Co., Ltd. (Japan). Cellotriose (Fine) was obtained from SEIKAGAKU Corporation, (Japan). 2-isocyanatoethyl methacrylate (2-IEM) monomer was obtained from Showa denko K. K. (Japan). Ammonium peroxodisulfate (APS) and N,N,N',N'-tetraethylethylenediamine (TEEDAm) were obtained from Wako Pure Chemical Industries, Ltd. (Japan). All were used without purification.

2.2 Measurements

NMR spectra were recorded with a JEOL JNM-EX400 Fourier transform NMR spectrometer in deuterium oxide using 3-trimethylsilyl-1-propanesulfonic acid sodium salt as an internal standard. IR spectra were taken with a Fourier transform JASCO FT/**IR-700** infrared spectrometer. The molecular weights of the polymers were determined by size exclusion chromatography (SEC) with a Shodex SB-806M HQ column (8.0 mm I. D. \times 300 mm) at 20 °C, using a water and 10 mM lithium bromide aqueous solution as eluent. The formation of aggregates in water was confirmed by transmission electron microscopy (TEM) with a JEOL JEM-2000FX Electron Microscope. The aqueous samples (1.0 mg/ml) were spotted onto carbon-coated copper grids (200 Å). The samples were air-dried at room temperature, after which they were stained with

2.0 wt% aqueous ammonium molybdenate. Dynamic light scattering (DLS) measurements were conducted using a Zeta Sizer Nano-ZS (Malvern Instruments) with a 4.0 mW He-Ne laser (wavelength, $\lambda = 633$ nm) at 20 °C. The aqueous samples were prepared to the same concentrations (1.0 mg/ml) for SEC analysis and TEM observations.

3. RESULTS AND DISCUSSION

3.1 Synthesis of Mono Vinyl Saccharide Monomers and their Polymerization

Cello-oligosaccharide-pendant vinyl monomers were synthesized without the use of any protecting group for the oligosaccharide (1, 2 and 3) component. 1-amino- β -saccharide (4, 5 and 6) was derived from the reducing terminals of oligosaccharide with ammonium hydrogen carbonate [17, 18]. The purity of 1-amino- β -saccharide was determined by HPLC analysis. The vinyl monomer was prepared with the coupling of 1-amino- β -saccharide with 2-isocyanatoethyl methacrylate (2-IEM). The typical ¹H-NMR spectra of (a) glucose, (b) glucose



Scheme 1. Synthetic route of the novel cellooligosaccharide pendant polymers.

monomer, and (c) obtained polymer are shown in Fig. 1. The spectrum of (a) shows two peaks at 4.6 to 5.3 ppm responsible for the α and β form structures signals at the C-1 position, i.e., at 4.66 ppm for the α form and 5.24 ppm for the β form. However, the two peaks at the C-1 position can not be observed in the spectrum of (b). On the other hand, 7 gives only the peak for the C-1 position at 4.82 ppm. These results suggest that only β structures were obtained from the conversion of the OH group to a vinyl group at the C-1 position. The typical synthetic procedure is shown in Scheme 1. The obtained vinyl monomers (7, 8, and 9) were polymerized in aqueous solution using ammonium peroxodisulfate as an initiator and N, N, N', N'-tetraethylethylenediamine as an accelerator. The obtained crude polymer was purified by dialysis in a dialysis tube and freeze-dried to give a white powdery polymer. Figure 1 (c) shows that the peaks at 4.6 and 5.3 ppm responsible for the vinyl structures signal have disappeared and that new peaks have appeared at 0.91, 1.15 and 2.0 ppm in response to polymerization. The synthesis that used glucose is discussed in the following paragraphs. Other oligosaccharide polymers were also prepared by the same method.

Glucose (10 g, 55.6 mmol) was dissolved in water (150 ml), and ammonium hydrogen carbonate (28.5 g) was added at intervals of 24 h, and was stirred at 37 °C for 2 days. The solution was diluted to 200 ml with water and concentrated to 50 ml. This procedure was repeated until disappearance of the ammonia odor. The purity of the freeze-dried product was 83.7 %. Glucosylamine (5.74 g, 26.8 mmol) was dissolved in a 1.0×10^{-3} M KOH aqueous solution (50 ml). 2-IEM (10.4 g, 67.0



Figure 1. ¹H-NMR spectra of (a) glucose, (b) glucose monomer, and (c) obtained polymer in deuterium oxide.

mmol) was added to the solution and stirred at 3 °C for 12 h, producing white precipitation as a by product. The precipitations were removed by filtration. The filtrate was washed with diethyl ether to remove traces of unreacted 2-IEM and then freeze-dried. The raw product was dissolved in water/methanol and recrystallized from acetone/diethyl ether. The crude product was isolated by column chromatography with Grand 120-SMC18-5 (20.0 mm I. D. × 250 mm) using water as an eluent, and the fraction was freeze-dried. The yield was 66.5 %. m.p. 105.5 - 107.2. IR (KBr, cm⁻¹): 3350, n_{O-H}; 2930, n_{C-H}; 1710, n_{C=0}(ester); 1650, n_{C=0}(amide); 1570, d_{N-H}.

¹H NMR (D₂O, d): 1.98 (s, 3H, CH₂=C(<u>CH₃</u>), 3.34-3.43 (m, 2H, from sugar), 3.49-3.58 (m, 2H, from sugar), 3.53 (t, 2H, CH₂), 4.26 (t, 2H, CH₂), 4.82 (d, 1H, H-1), 5.73 (s, 1H, <u>CH₂</u>=C(CH₃) (cis)), 6.15 (s, 1H, <u>CH₂</u>=C(CH₃) (trans)). Elemental Anal. Calcd. for $C_{19}H_{32}N_2O_{13}$: C, 54.9; H, 7.09; N, 9.85. Found: C, 54.9; H, 7.02; N, 9.85.

The obtained 7 (0.76 g, 1.61 mmol) was dissolved in water (5.0 ml). Air was degassed from the solution by dry nitrogen gas and APS (3.66 mg, 5.85 mmol) and TEEDAm (27.8 mg, 0.161 mmol) were added. The mixture was stirred at 0 $^{\circ}$ C under a nitrogen gas atmosphere for 3 h. The obtained crude polymer was purified by dialysis in a dialysis tube for 3 days and then freeze-dried to give a white powdery polymer. The yield was 74.5 %.

3.2 Molecular Weight Determination of the Polymers

The molecular weights of the obtained polymers were determined by size exclusion chromatography (SEC) using a Shodex SB-806M HQ column with an exclusion molecular weight value (M_{lim}) of 2.0 × 10⁷. The SEC calibration curve was prepared by a pullulan standard (Shodex STANDARD P-82) that is fairly stable in aqueous solution [19, 20]. Figure 2 shows the typical SEC chromatograms of pullulan standards and the obtained polymer, respectively, using a water and lithium bromide (LiBr) aqueous solution as an eluent. The pullulan do not form the an aggregate by inter- and intramolecular hydrogen bonding. As expected, no significant changes in SEC behavior occurred for the pullulan standard using the modified eluent, i.e., the molecular weight distribution and elution time because

of a lack of aggregation in the solvent. In contrast, the elution of polymers in the modified eluent occurred much later than that of polymers using water as an eluent. The SEC behaviors for polymers indicated that the domain size of polymers in water is huge. We suspected that the polymers formed a huge aggregate caused by inter- and intramolecular hydrogen bonding among oligosaccharide groups in the side chain in water. As a result, the corresponding weight-average molecular weights of **10**, **11** and **12** were estimated at ca. 0.78 × 10^6 , 3.14×10^6 and 3.87×10^6 , as determined by SEC using a modified eluent. The difference in their







Figure 3. TEM images of the obtained oligosaccharide pendant polymers in an aqueous solution. (a) compound **10**, (b) compound **11**, and (c) compound **12**. Initial concentration : 1.0 mg/ml



Figure 4. DLS size distribution of the obtained oligosaccharide polymer aggregates in the aqueous solution. (a) number base-size distribution, (b) volume base-size distribution

molecular weights was probably caused by the specific conformation resulting from inter- and intramolecular hydrogen bonding among the side chains in water.

3.3 Aqueous Properties of Polymers

The formation of aggregates in aqueous solutions was confirmed by transmission electron microscopy (TEM). Figure 3 shows TEM images of the obtained polymers. The images of the obtained polymers demonstrated spherical nano-sized particles. In contrast, the general water-soluble vinyl polymers such as poly(2hydroxyethyl methacrylate), poly(vinyl alcohol), and poly(acrylamide) were not able to form aggregates such as the spherical nano particles [21, 22]. It therefore, it appeared that the obtained polymers possessed different properties from common water-soluble vinyl polymers in an aqueous solution. In addition, the size distribution of the aggregates in the aqueous solution was determined by dynamic light scattering (DLS). Figure 4 (a) shows the number base-size distribution of polymers estimated by DLS. The size of the nano particles was distributed from around 10 nm to 20 nm. However, the nano particles with diameters of approximately 30 nm or more observed in the TEM images were not including to the number base-size distribution. Since the number of larger particles was small, as shown in the TEM image, the volume base-size distribution of polymers was plotted simultaneously. As expected, it is evident that at least two populations of aggregate domains coexist under this condition, as shown in Fig. 4 (b). As a result, the aggregation size depends on the kinds of oligosaccharides in the side chain, and the nano particles with diameters of approximately 30 nm or more observed by TEM were detected on the volumebase size-distribution. The DLS measurement data

corresponded quite well to the abovementioned TEM observation. These results suggest that it is most stable for the obtained polymer to form spherical nano particles ranging from around 10 - 40 nm in aqueous solution. The results of the TEM observations and the DLS measurements confirm that the obtained polymers form nano-spherical particles in an aqueous solution by self-aggregation, which dose not usually occur with water-soluble polymers.

4. CONCLUSION

We were able to synthesize novel oligosaccharide pendant vinyl polymers, and to observe the conformations of the obtained polymers in an aqueous solution. These polymers formed huge aggregates in aqueous solution, taking on the shape of spherical nano particles. It should be emphasized that the obtained nano particles were dispersed in an aqueous solution.

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