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Synthesis of polystyrene microgel with glucose as hydrophilic segment via controlled free-radical polymerization

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Abstract

4-Vinylbenzyl glucoside peracetate (**1**) was copolymerized with divinylbenzene (DVB) using 1-phenyl-1-(2′,2′,6′,6′-tetramethyl-1′-piperidinyloxy)ethane (**2**) as an initiator in *m*-xylene at 138 °C for 20 h ([DVB]/[**2**] = 28; [DVB] = 0.62 mol L^{-1}). The copolymerizations were performed using the mole fraction of **1** in the total feed of **1** and DVB (F_1: [**1**]/([**1**] + [DVB]) ranging from 0.11 to 0.38 that produced the polystyrene (PSt) microgel with acetyl glucose, **3**, in 46–53% yields. Dynamic laser light scattering (DLS) measurements showed that **3** was stably suspended in toluene as particles with average diameters (*d*'s) ranging from 12 to 22 nm. A static laser light scattering (SLS) measurement gave the average molar mass, $M_{w,SLS}$, of **3** that ranged from 9.69×10^4 to 6.96×10^5. The numbers of the **1**, **2**, and DVB units in **3** (N_1, N_2, and N_{DVB}, respectively) were from 111 to 238, from 17 to 208, and from 350 to 4510, respectively. The deacetylation of **3** was achieved by treatment with sodium methoxide in dry 1,4-dioxane to produce the PSt microgel with glucose as the hydrophilic segment, **4**. The solubilities of **4** in toluene, $CHCl_3$, THF, 1,4-dioxane, pyridine, DMF, DMSO, and H_2O, and the mixture of H_2O and 1,4-dioxane were examined, indicating that a hydrophilic property had been effectively introduced into **4**.

Keywords: Glycoconjugated polymer; Microgel; Controlled free-radical polymerization

1. Introduction

Saccharide is one of the important biological compounds, which is used as an economic starting material for polyester [1] and the chiral source for optically active polymers [2] in polymer chemistry. In addition, the highly water-soluble property of the saccharide is a useful tool for the hydrophilic segment to produce an amphiphilic polymer, and thus various types of living polymerization techniques using vinyl saccharides have been developed for the synthesis of block copolymers with a pendant saccharide moiety, i.e. the glycoconjugated block copolymers, which have exhibited amphiphilic properties such as a surface activity and a self-assembling property [3–15]. Furthermore, of great interest is to design and synthesis of a three-dimensional macromolecular architecture with the saccharide from the viewpoint of constructing new amphiphilic materials. Previously, we reported that a star-shaped polymer with a glycoconjugated core was prepared via the nitroxide-mediated living radical polymerization [16]. Recently, Müller and coworkers reported that hyperbranched glycopolymers were synthesized via the self-condensing atom transfer radical copolymerization of a sugar-carrying acrylate [17].

Staudinger and Husemann first described a 'microgel' as one having a three-dimensional macromolecular architecture [18], which is defined today as a cross-linked polymer particle being able to exist as a stable solution in appropriate solvents [19,20]. Micogels are now very important as coatings in industry and also used as substrates for biomedical and diagnostic purposes, monolithic stationary phases in liquid chromatography, and a support for catalysts. In addition, microgels are traditionally synthesized by the free-radical copolymerization of monovinyl monomers and divinyl monomers in suitable solvents under dilute conditions, while Solomon et al. showed that the living free radical

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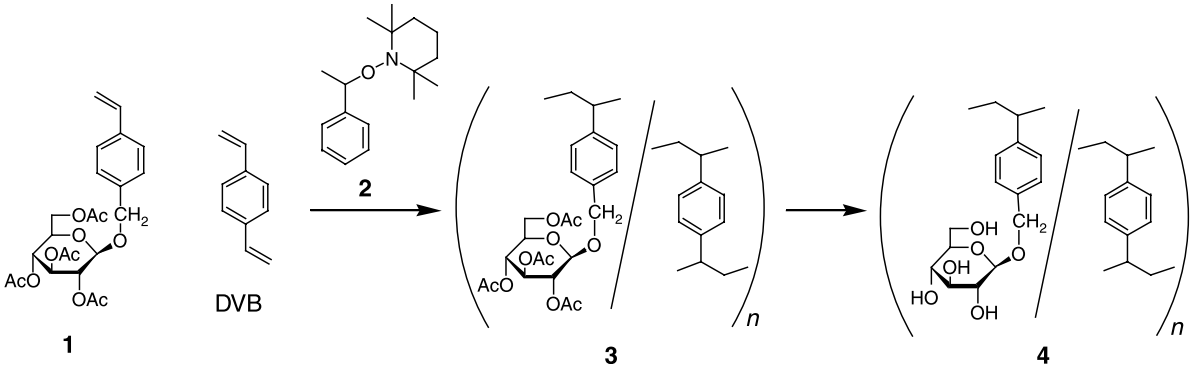

Scheme 1. Copolymerization of vinyl saccharide **1** with DVB using initiator **2** to afford PSt microgel with acetyl glucose, followed by deacetylation generating PSt microgel with glucose as hydrophilic segment.

polymerizations of *t*-butylstyrene using divinylbenzene as a linking agent was an efficient method for the controlled synthesis of cross-linked microgels [21,22]. To date, however, there have been few reports on the synthesis of microgel derived from vinyl saccharides. Thus, of interest is to realize the synthesis of a poly(vinyl saccharide) microgel, as a new glycoconjugated three-dimensional macromolecular architecture, using such developed methods. In this paper, we describe the synthesis of the poly(4-vinylbenzyl glucoside) microgel via copolymerization of 4-vinylbenzyl glucoside peracetate (**1**) with divinylbenzene (DVB) using 1-phenyl-1-(2′,2′,6′,6′-tetramethyl-1′-piperidinyloxy)ethane (**2**) as the initiator in *m*-xylene (Scheme 1). After the deacetylation of the PSt microgel with acetyl glucose (**3**), the solubility of the microgel **4** was examined using appropriate solvents in order to clarify the effect of the glucose unit as the hydrophilic segment on the amphiphilic property of **4**.

2. Experimental

2.1. Materials and measurements

Vinyl saccharide **1** and the initiator **2** were prepared according to literature procedures [23,24]. DVB (Aldrich, tech., mixture of isomers, 80%) and *m*-xylene (Kanto Chemical Co., >99.0%) were distilled just before use. Dry 1,4-dioxane (Kanto Chemical Co., Japan, >99.5%), sodium methoxide (Wako Pure Chemical Industries, Japan, 28% in methanol), and spectroscopy grade chloroform (Merck) were used without further purification. A seamless cellulose tube (UC24-32-100) was obtained from Viskase Sales Co.

The ^{1}H NMR and ^{13}C NMR spectra were recorded using a JEOL JNM-GX270 instrument. Optical rotations were measured using a Jasco DIP-1000 digital polarimeter. The size exclusion chromatography (SEC) was performed at 40 °C in chloroform (1.0 mL min^{-1}) using a Jasco GPC-900 system equipped with a Shodex KF-804L column (linear, 8 mm× 300 mm) and a Shodex KF-805L column (linear, 8 mm× 300 mm). The weight-average molecular weight ($M_{w,SEC}$) and polydispersity (M_w/M_n) of the polymers were calculated on the basis of a PSt calibration. The dynamic laser light scattering (DLS) measurement was performed in toluene at 22 °C using an Otsuka Electronics DLS-7000 light scattering spectrophotometer equipped with an argon ion laser (λ=488 nm). A scattering angle of 90° was used in this study. The static laser light scattering (SLS) measurement was performed in toluene at 25 °C on an Otsuka Electronics DLS-7000 light scattering spectrophotometer (λ=633 nm; four-point measurements; c= 2–10 mg/mL). The refractive index increment (dn/dc) was measured in toluene at 25 °C using an Otsuka Electronics DRM-1021 double beam-differential refractometer (λ= 633 nm).

2.2. Copolymerization and calculation

A mixture of **1** (815 mg, 1.75 mmol), **2** (26.1 mg, 0.100 mmol), and DVB (457 mg, ca. 2.8 mmol) in *m*-xylene (4.5 mL) was degassed by three freeze/thaw cycles, sealed under argon, and heated at 138 °C for 20 h. After cooling in liquid nitrogen, the mixture was diluted with chloroform (15 mL), and then precipitated in methanol (ca. 1 L). The precipitate was purified by reprecipitation using chloroform–methanol and dried in vacuo to give **3**-**V** as a white solid. Yield: 586 mg (46.0% based on the total feed of **1**, **2**, and DVB). $M_{w,SEC}=2.87\times10^4$, $M_w/M_n=2.28$. $M_{w,SLS}=9.69\times10^4$, dn/dc=0.07815 mL g^{-1}. $[\alpha]_D^{23}=-25.8°$ (c 1.0, $CHCl_3$).

The weight-fractions of the **1**, **2**, and DVB units (W_1, W_2, and W_{DVB}, respectively) in **3** were calculated as follows. The $[\alpha]_D^{23}$ of −26.8°(c 1.0, $CHCl_3$) for **3**-**V** was divided by that of −50.4° for poly(4-vinylbenzyl glucoside peracetate), poly-**1**, giving the W_1 value of 0.531. The result that **2** (26.1 mg) was quantitatively consumed gave the W_2 of 0.045. The relationship ($W_1+W_2+W_{DVB}=1$) gave the W_{DVB} of 0.424. The W_1, W_2, and W_{DVB} values were multiplied by $M_{w,SLS}$ of 9.69×10^4 and then divided by the molecular weights of the corresponding units, i.e. W_1M_w 464^{-1}, W_2M_w 261^{-1}, and $W_{DVB}M_w$ 130^{-1}. These values are equal to the numbers of the **1**, **2**, and DVB units (N_1, N_2, and N_{DVB}) in **3**-**V** of 111, 17, and 350, respectively.

2.3. Deacetylation

Typically, a solution of **3**-**V** (300 mg) in dry 1,4-dioxane (5 mL) was added to a dry 1,4-dioxane solution containing

2 wt% sodium methoxide (2 mL). The reaction mixture was stirred for 24 h at room temperature, and then poured into water (ca. 80 mL). The mixture was transferred to a cellulose tube and dialyzed for 2 days against distilled water, followed by freeze–drying to yield **4-V** as a white solid. Yield: 250 mg (97.6%).

3. Results and discussion

3.1. Synthesis and characterization of polystyrene microgels with acetyl glucose

The synthetic procedure is based on the 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated radical polymerization [25]. 4-Vinylbenzyl glucoside peracetate (**1**) was copolymerized with divinylbenzene (DVB) using 1-phenyl-1-(2′,2′,6′,6′-tetramethyl-1′-piperidinyloxy)ethane (**2**) as an initiator in *m*-xylene, as shown in Scheme 1. The copolymerizations were performed at 138 °C using constant feeds for DVB and **2** ([DVB]/[**2**] = 28; [DVB] = 0.62 mol L^{-1}), and the mole fraction of **1** in the total feed of **1** and DVB (F_1: [**1**]/([**1**] + [DVB]) was varied from 0.11 to 0.38 (Table 1). After 20 h, the homogeneous reaction mixtures were purified by reprecipitation, affording products in the yields of 46–53% which were based on the total feed of the starting materials (Table 1). Only DVB was polymerized with **2** to give poly(DVB), a controlled product, in a 59% yield. Although the filtration of **3** was performed using a 0.5 μm PTFE membrane filter, there was little or no weight loss [26], indicating that the products contained no highly crosslinked species, such as a macrogel. The good solvents for the products were similar to those for the controlled product, e.g. toluene, $CHCl_3$, THF, 1,4-dioxane, pyridine, and DMF (Table 2).

In general, the crosslinked polymers are insoluble in solvents. However, microgels exist as stable solutions in appropriate solvents, thus being able to be characterized by standard techniques available for soluble macromolecules. Earlier, the product was characterized by size exclusion chromatography (SEC) based on linear PSt standards, because the weight-average molecular weights ($M_{w,SEC}$) and polydispersity (M_w/M_n) should provide information about the apparent hydrodynamic volumes and their distributions, respectively (Fig. 1). The SEC traces of the products generally showed bimodal peaks with the $M_{w,SEC}$'s and the M_w/M_n's that ranged from 28,700 to 53,500 and from 2.28 to 5.52, respectively. These are low values compared to the $M_{w,SEC}$ of 118,000 and the M_w/M_n of 12.4 of the poly(DVB) obtained from the multimodal SEC trace in Fig. 1(f). Dynamic laser light scattering (DLS) measurements of the products in toluene solution showed a very strong scattering intensity and indicated that the products were stably suspended as particles with the average diameters (*d*'s) ranging from 12 to 22 nm (Table 1). Thus, the product was assignable to the polymeric particle being able to produce stable solutions, i.e. the microgel. The DLS analysis also indicated that the product possessed a size comparable to the statistical dimensions of noncross-linked macromolecules (10–100 nm), thus being able to exist as stable solutions in appropriate solvents.

Table 1
Synthesis of microgel **3** by the copolymerization of **1** with DVB using initiator **2**

F_1[a]	Product	Yield (%)	d[b] (nm)	$[\alpha]_D$[c] (°)	$M_{w,SLS}$[d]	d*n*/d*c* (mL g^{-1})	Number of the unit		
							N_1	N_2	N_{DVB}
0.11	**3-I**	53	21 ± 7	−8.0	696,000	0.11520	238	208	4510
0.20	**3-II**	51	22 ± 6	−14.6	510,000	0.10145	318	124	2640
0.27	**3-III**	50	20 ± 7	−20.1	299,000	0.09107	257	63	1440
0.33	**3-IV**	52	22 ± 8	−23.6	186,000	0.08424	185	32	780
0.38	**3-V**	46	12 ± 4	−26.8	96,900	0.07815	111	17	350
–[e]	Poly(DVB)	59	54 ± 14		1,010,000	0.12906		354	7050

[a] Mole fraction of **1** in the total feed of **1** and DVB ([**1**]/([**1**] + [DVB]).
[b] Particle diameter determined by DLS measurement in toluene at 22 °C.
[c] Measured in $CHCl_3$ at 23 °C (*c* 1.0).
[d] Determined by SLS measurement in toluene at 25 °C.
[e] Control.

Table 2
Solubility of **4** in various solvents

Sample	Toluene	$CHCl_3$	THF	1,4-Dioxane	Pyridine	DMF	DMSO	H_2O
4-I	±	+	+	+	+	+	−	−
4-II	±	±	±	+	+	+	−	−
4-III	−	−	−	±	+	+	±	−
4-IV	−	−	−	±	+	+	+	−
4-V	−	−	−	±	+	+	+	−
Poly(DVB)[a]	+	+	+	+	+	+	−	−
Glucose[a]	−	−	−	−	+	+	+	+

+, Clear solution; ±, suspension; −, precipitate.
[a] Control.

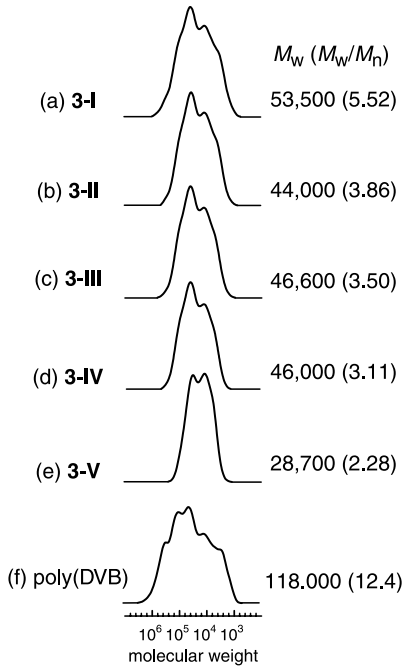

Fig. 1. SEC traces of (a) **3-I**, (b) **3-II**, (c) **3-III**, (d) **3-IV**, (e) **3-V**, and (f) poly(DVB).

Fig. 2(a) shows the ^{1}H NMR spectrum of the product. The signals due to the aromatic protons (5.9–8.0 ppm) and the methine and methylene protons in the polymer backbone (0.5–3.5 ppm) appeared. These signals are extremely broadened compared to those for poly(4-vinylbenzyl glucoside peracetate), poly-**1** (Fig. 2(b)), suggesting that the intramolecular mobility of the protons on the polymer backbone is suppressed by crosslinking. In Fig. 2(a), the signals derived from the **1** units appeared, i.e. at 3.5–5.8 ppm, due to the methine and methylene protons of the saccharides and at 1.8–2.5 ppm, due to the acetyl protons. Notably, the signals due to the **1** unit were also broadened, suggesting that the **1** unit exists in the neighborhood of the microgel backbone. The specific rotations ($[\alpha]_D^{23}$, *c* 1.0 $CHCl_3$) of the products ranged from −8.0 to −26.8°, whose signs were consistent with that of poly-**1** (−50.4°). Hence, the product was assigned to the PSt microgel with acetyl glucose, **3**.

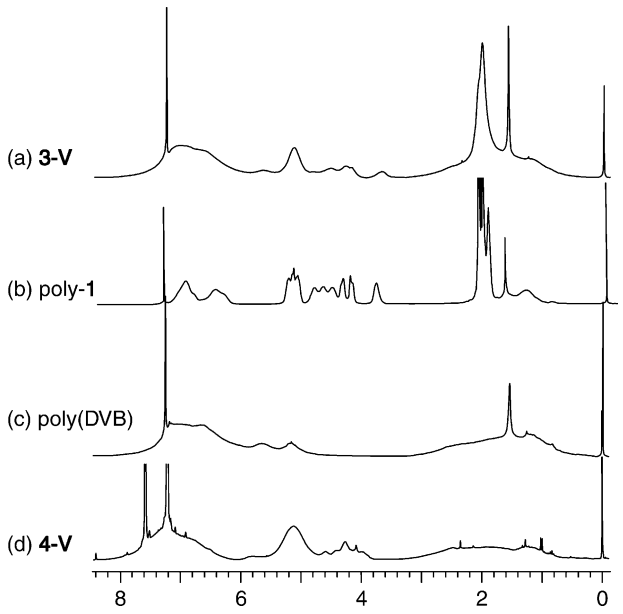

Fig. 2. ^{1}H NMR spectra of (a) **3-V** in $CDCl_3$, (b) poly-**1** in $CDCl_3$, (c) poly(DVB) in $CDCl_3$, and (d) **4-V** in pyridine-d_5.

Table 1 summarizes the synthesis of **3-I**, **II**, **III**, **IV**, and **V** which correspond to the products obtained by the copolymerizations with the F_1's of 0.11, 0.20, 0.27, 0.33, and 0.38, respectively. The $[\alpha]_D^{23}$ values suggested that the contents of the chiral saccharide units in **3** increased with the increasing F_1's. In order to elucidate the structure of **3** in more detail, a static laser light scattering (SLS) measurement was carried out. Table 1 lists the average molar masses, $M_{w,SLS}$'s, of **3** ranging from 96,900 to 696,000, which were determined using the respective refractive index increments, d*n*/d*c*'s, ranging from 0.07815 to 0.11520. We calculated the N_1, N_2, and N_{DVB} values, which means the apparent numbers of the **1**, **2**, and DVB units in **3** estimated from the respective $M_{w,SLS}$'s, real yields, and specific rotations (Section 2). The N_1, N_2, and N_{DVB} of **3** ranged from 111 to 238, from 17 to 208, and from 350 to 4510, respectively (Table 1). In general, the N_{DVB} of **3** decreased with the increasing F_1. This can be explained as follows: Initially, **1** is copolymerized with DVB by **2** to afford short copolymers. The pendant vinyl groups in the copolymer then add to the terminal alkoxyamine in another copolymer chain. The copolymerization and crosslinking continuously proceed to form **3**. The crosslinking should be hindered when the bulky **1** unit exists in the neighborhood of the pendant vinyl groups, resulting in decreased N_{DVB} with the increasing F_1.

These characterizations and calculations revealed more important information, i.e. **3** was shown to possess a large number of acetyl groups (potentially equal to hydroxyl groups) available in limited space. For examples, **3-V** possesses 111 glucose residues, which is equal to more than 400 acetyl groups, with the small *d* values of 12 nm. Such a macromolecular architecture, which has numerous functional groups on a three-dimensional structure with a nano-scale size, is attractive for drug targeting applications [27,28].

3.2. Polystyrene microgel with glucose as hydrophilic segment

The deacetylation of the microgel **3** was achieved by treatment with sodium methoxide in dry 1,4-dioxane to produce the PSt microgel with glucose as the hydrophilic segment, **4**, as a white solid (Scheme 1). Fig. 2(d) shows the ^{1}H NMR spectrum of **4** in pyridine-d_5. The signals due to saccharides (3.8–6.0 ppm) appeared, whereas the large signals due to the acetyl protons (1.8–2.5 ppm) disappeared. The signals due to the aromatic protons (6.0–8.0 ppm) and the methine and methylene protons (0.5–3.5 ppm) were difficult to detect due to the lack of intramolecular mobility. We examined the solubility of this new type of the glycoconjugated polymeric architecture. As expected, **4** produced clear solutions in the good solvents for both the PSt microgel and saccharides, such as pyridine and DMF. In the good solvents

Table 3
Solubility of **4** (and glucose as controls) in H_2O/1,4-dioxane

Sample	10/0	9/1	8/2	7/3	6/4	5/5	4/6	3/7	2/8	1/9	0/10
4-I	−	−	−	±	±	±	±	±	±	±	+
4-II	−	−	−	±	±	±	±	±	±	±	+
4-III	−	−	−	±	±	±	±	±	±	±	±
4-IV	−	−	−	±	±	±	±	±	+	+	±
4-V	−	−	−	±	±	±	±	+	+	+	±

+, Clear solution; ±, suspension; −, precipitate.

for the PSt microgel, but poor ones for the saccharides, such as toluene, $CHCl_3$, THF, and 1,4-dioxane, almost all the samples produced a suspension or precipitate (Table 2). Reversibly, in the poor solvents for the PSt microgel, but good ones for the saccharides, such as DMSO, the solubility improved with the increasing saccharide contents. Thus, the hydrophilic property was effectively introduced into **4**. Interestingly, **4** with a high content of saccharides gave clear solutions of 1,4-dioxane containing H_2O (Table 3). For example, **4-V** gave clear solution in 1,4-dioxane containing 10–30% H_2O. Consequently, the hydrophilic property [29] was successfully introduced into the highly hydrophobic PSt microgel by utilizing saccharides, which has the potential as a special coating, substrates for biomaterials, and a support for catalysts in aqueous media.

4. Conclusions

The copolymerization of the vinyl saccharide with DVB using the living free radical polymerization technique afforded a new class of glycoconjugated macromolecular architectures, i.e. the PSt microgel with glucose as the hydrophilic segment. The microgel possessed a large number of hydroxyl groups available in the limited space of the hydrophobic microgel backbone, being applicable to special coatings, substrates for biomaterials, and supports for catalysts in aqueous media.

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