A Well-Defined Novel Aldehyde-Functionalized Glycopolymer: Synthesis, Micelle Formation, and Its Protein Immobilization

Nai-Yu Xiao, An-Long Li, Hui Liang, and Jiang Lu*

Department of Polymer and Materials Science, School of Chemistry and Chemical Engineering, SunYat-sen (Zhongshan) University, Guangzhou 510275, China

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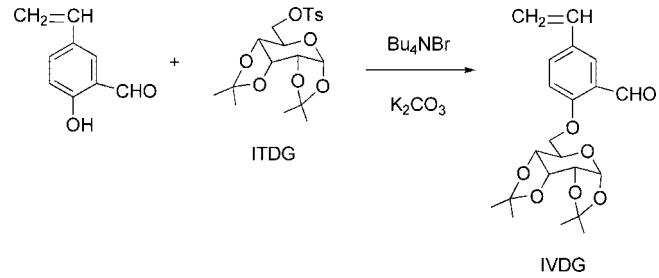
ABSTRACT: A new aldehyde-functionalized glycomonomer, 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′- formyl-4′-vinylphenyl)-D-galactopyranose (IVDG), was designed and prepared. The "living"/controlled radical polymerization of IVDG was successfully achieved using 2,2′-azobis(isobutyronitrile) as the initiator and 1-phenylethyl dithiobenzoate as the reversible addition−fragmentation chain transfer (RAFT) agent at 60 °C in tetrahydrofuran. The polymerization followed first-order kinetics, the number-average molecular weight of the obtained polymers increased in direct proportion to the monomer conversion, and the molecular weight distribution was narrow (polydispersity index <1.1). Removal of protective isopropylidene groups from the sugar residue in polyIVDG was carried out quantitatively using 88% formic acid at room temperature, yielding a novel amphiphilic polymer containing both galactopyranose and aldehyde functionalities. These amphiphilic polymers self-assembled into well-defined aldehyde-bearing polymeric micelles in aqueous solution without recourse to any surfactant. The size of the micelles increased almost linearly with the molecular weight of polyIVDG precursor, which could be controlled directly via the aforementioned RAFT polymerization process. Protein-bioconjugated nanoparticles were also successfully prepared by the immobilization of bovine serum albumin (as a model protein) onto the aldehyde-functionalized micelles.

Introduction

Synthetic polymers with pendent saccharides, also known as glycopolymers, have received more and more attention as smart artificial materials for various biological and biomedical applications.[1] This is mostly due to the expectation that glycopolymers might be able to mimic or even exceed the performance of natural polysaccharides in some specific applications.[2] Therefore, considerable effort has been made to the synthesis of saccharide-functionalized vinyl monomers (glycomonomers) and their polymers.[1,3] Construction of polymers with highly reactive functionalities is an emerging research area in modern polymer chemistry.[4] The selection of polymers with particular kinds of reactive groups depends on their intended applications. Polymers bearing aldehyde groups, however, are versatile and convenient, especially for medical and biological applications.[5] Aldehyde groups are able to react under mild conditions with primary amino groups and with hydrazines forming the corresponding Schiff base and hydrazone linkages, respectively. The polymers containing aldehyde groups can thus be used as carriers for the direct immobilization of catalysts, enzymes, drugs, and various biopolymers such as proteins and peptides.[6–9]

Herein we are interested in designing a new glycomonomer, 1,2:3,4-di-*O*-isopropylidene-6-*O*-2′-formyl-4′-vinylphenyl)-D-galactopyranose (IVDG, Scheme 1), for the synthesis of a polymer containing both galactopyranose (in an acetal-protected form) and aldehyde functionalities, in which the sugar moieties can render the polymer biocompatible and hydrophilic while aldehyde groups may offer a very simple, mild, and firm combination for biomolecules immobilization. The pendent galactopyranose moieties regenerated after deprotection provide high water solubility for the macromolecule amphiphile, and this amphiphilic polymer should self-assemble into micellar aggregates with aldehyde anchoring groups in aqueous solution without recourse to any surfactant. The resulting micelles may be used as nanocarriers for covalent immobilization of proteins and enzymes to obtain biomolecule−nanosphere systems, which have found wide applications in the biosensor, extracorporeal therapy, and bioseparation.[10]

Scheme 1



In addition to the functional groups in a glycopolymer, control of the macromolecular length has proved essential to enable sophisticated functions required for a given application.[2,11,12] For this reason various kinds of living polymerization strategies have been applied to prepare well-defined glycopolymers.[1,13] Examples include living ionic polymerizations,[14–19] ring-opening polymerization,[20–22] and ring-opening metathesis polymerization.[23] Recently, "living"/controlled radical polymerizations including nitroxide-mediated polymerization,[24–28] atom transfer radical polymerization,[29–31] and reversible addition−fragmentation chain transfer (RAFT) polymerization[32–36] have proven to be versatile and robust methods to synthesize glycopolymers of controlled dimension. Owing to the high compatibility with functional groups and the relatively mild reaction conditions, the RAFT technique was selected for the molecular weight control of the polymerizations in this study.

Therefore, we report the first example of the synthesis of a new aldehyde-functionalized glycomonomer (IVDG, Scheme 1) and its "living"/controlled RAFT radical polymerization in the present paper. The possibility of self-assembly into micellar aggregates from the amphiphilic polymers, obtained by the hydrolysis of polyIVDG precursors, as well as their potential application as a matrix for protein immobilization was also investigated by transmission electron microscopy (TEM) and

* Corresponding author: Tel +86-20-84037562; Fax +86-20-84112245; e-mail gaofenzi@mail.sysu.edu.cn.

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dynamic light scattering (DLS). Well-defined aldehyde-functionalized polymeric micelles with controllable size and high capacity to immobilize proteins (BSA as a model protein) were prepared from the polyIVDG precursors whose molecular weight influenced directly the size of the micelles and could be controlled facilely via the RAFT polymerization process. Micelle formation from amphiphilic block or graft copolymers is well-documented;[37,38] however, micelles formed by amphiphilic homopolymers were scarcely reported.

Experimental Section

Materials. 1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose (97%, Aldrich), *p*-toluensulfonyl chloride (TsCl; Acros, 99%), K_2CO_3 (China National Medicines Shanghai Co., 99%), formic acid (China National Medicines Shanghai Co., 88%), tetrabutylammonium bromide (Bu_4NBr; Acros, 99%), dimethyl sulfoxide (DMSO; China National Medicines Shanghai Co., 99%), and bovine serum albumin (BSA; Acros, protease free) were all used as received. Tetrahydrofuran (THF; China National Medicines Shanghai Co., 99%) was refluxed with sodium chips under N_2 until dry and freshly distilled before use. Pyridine and *N*,*N*-dimethylformamide (DMF) (both from China National Medicines Shanghai Co., 99%) were distilled under reduced pressure from CaH_2 before use. 2,2′-Azobis(isobutyronitrile) (AIBN; China National Medicines Shanghai Co., 99%) was purified by recrystallization from ethanol. 1-Phenylethyl dithiobenzoate (PEDB),[39] 1,2:3,4-di-*O*-isopropylidene-6-*O*-(*p*-toluenesulfonyl)-D-galactopyranose (ITDG),[40] and 2-hydroxy-5-vinylbezaldehyde[41] were synthesized according to literature procedures.

Synthesis of 1,2:3,4-Di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)-D-galactopyranose (IVDG). IVDG was synthesized by the reaction of 2-hydroxy-5-vinylbezaldehyde with ITDG as below. A solution of ITDG (5.2 g, 20 mmol) in DMF (50 mL) was added dropwise to a stirred mixture of 2-hydroxy-5-vinylbezaldehyde (2.96 g, 20 mmol), K_2CO_3 (2.76 g, 20 mmol), and Bu_4NBr (0.6 g, 2 mmol) in DMF (100 mL) at 130 °C. When no more starting material was detected by thin-layer chromatography, the reaction mixture was cooled, concentrated under reduced pressure, and then poured into water. The organic material was extracted with ether. The extract was washed with water, dried over Na_2SO_4, and then evaporated to dryness. The residue was purified by silica gel chromatography eluted with *n*-hexane and then ethyl acetate/*n*-hexane (1:10 v/v) to give IVDG as a white powder solid (4.68 g, 60%); mp = 97−98 °C. ^{1}H NMR ($CDCl_3$), δ (TMS, ppm): 10.48 (s, 1H, aldehyde proton), 7.86 (d, J = 2.1 Hz, 1H, 3′-aromtic proton), 7.58 (dd, J_1 = 2.1 Hz, J_2 = 8.4 Hz, 1H, 5′-aromtic proton), 7.01 (d, J = 8.7 Hz, 1H, 6′-aromtic proton), 6.67 (dd, J_1 = 10.8 Hz, J_2 = 17.4 Hz, 1H, =CH−), 5.72 (d, J = 17.4 Hz, 1H, *cis*-CH_2=), 5.24 (d, J = 11.1 Hz, 1H, *trans*-CH_2=); 5.58, 4.70, 4.38, and 4.28 (7H, sugar moiety), 1.60–1.36 (m, 12H, CH_3). ^{13}C NMR ($CDCl_3$), δ (TMS, ppm): 189.62 (1C, aldehyde carbon), 160.72, 133.49, 131.04, 125.92, 125.19, and 113.71 (6C, aromtic carbon); 135.34 (1C, =*C*H−), 113.46 (1C, *C*H_2=); 109.83, 109.05 (2C, $(CH_3)_2C(OCH-)_2$); 96.56, 71.30, 70.96, 70.83, 68.15 and 66.70 (6C, sugar moiety); 26.38–24.79 (4C, *C*H_3). Anal. Calcd for $C_{21}H_{26}O_7$: C 64.62; H 6.67. Found: C 64.63; H 6.78. FAB MS: *m*/*z* calcd for $C_{21}H_{26}O_7$ 390.43; found 390 (M^+).

RAFT Polymerization of IVDG. For a typical RAFT polymerization, a stock mixture in THF (12 mL) comprising IVDG (3.7 g, 9.6 mmol), PEDB (18.6 mg, 72 umol), and AIBN (7.4 mg, 45 umol) was prepared in a N_2-filled dry glovebox. Aliquots (2.0 mL) were removed and transferred to ampules, degassed by three freeze–pump–thaw cycles, sealed, and heated at 60 °C. After the predetermined intervals, the sealed ampules were cooled in an ice bath, and the reaction mixtures were diluted with THF and then poured into a large amount of methanol. The precipitated polymers were washed with methanol and dried to constant weight under vacuum at 40 °C; the overall monomer conversions were determined gravimetrically.

Deprotection of PolyIVDG. Removal of the protective isopropylidene groups from the sugar residue was achieved by the conventional method.[42] For a typical example, polyIVDG [100 mg, M_n (number- average molecular weight) ∼ 13 000; M_w (weight-average molecular weight) ∼ 14 170] was dissolved in 88% formic acid (12 mL) and stirred for 24 h at room temperature. The solution was then diluted by adding 5 mL of water and stirred for another 3 h. The resulting solution was dialyzed against distilled water for 2 days using a semipermeable membrane (cutoff molecular weight 1000). After lyophilization, 70 mg of the polymer with galactopyranose moieties was obtained as white powders (yield 88%). The ^{1}H NMR analysis for the resulting deprotected polymer indicated the complete hydrolysis of the protecting acetal groups (see Results and Discussion section).

Preparation of Polymer Micelles. The deprotected polymers (58 mg) were dissolved in DMSO (2 mL). Deionized water (36 mL) was then added dropwise with stirring. The resulting colloidal solution was dialyzed against distilled water for 4 days using a semipermeable membrane (cutoff molecular weight 1000) to remove the organic solvent DMSO.

BSA Covalent Immobilization to Polymer Micelles. The polymer micelle solution (6.5 mL, at a polymer concentration of l.5 g/L) was mixed with 0.5 mL of BSA solution in water (4 g/L). The mixture was stirred at 25 °C for 8 h. At the end of the experiment, the immobilized protein was separated by three cycles of centrifugation (18 000 rpm for 1 h at 4 °C) and reconstituted with deionized water. The supernatants were analyzed for the immobilized BSA concentration by the standard Bradford protein assay method.[43]

Measurements. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in $CDCl_3$ or DMSO-d_6 at room temperature on a Varian Unity Inova 300 spectrometer. Elemental analysis was determined on a CHNS-Vario elemental analyzer. FAB mass measurement was carried out on a VG ZAB-HS spectrometer. Thermogravimetric analysis (TGA) was measured on a Netzsch TG/209 under nitrogen at a heating rate of 10 °C/min. Differential scanning calorimetry (DSC) was performed on a Netzsch DSC/204. Samples were heated from 25 to 200 °C at a rate of 10 °C/min, kept for 5 min, and cooled at a rate of 10 °C/min. The data collection was carried out on the second heating process, and the glass transition temperature (T_g) was taken to be the midpoint (the temperature corresponding to half of the endothermic shift). The onset temperature was determined at the point which the extrapolated initial baseline on the low-temperature side of the curve is intersected by the tangent to the curve at the point of inflection. The calorimeter was calibrated with an indium standard.

M_n, M_w, and molecular weight distribution (M_w/M_n) were measured by gel permeation chromatography (GPC) against polystyrene standard in THF at a flow rate of 1.0 mL/min at 35 °C on three Waters Styragel columns (measurable molecular weight range: 100–5000, 500–30 000, and 5000–600 000) connected to a Waters 1515 pump and a Waters 2414 refractive index detector. The precipitated polymers were used for GPC analysis.

Matrix-assisted laser desorption−ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was performed on a Bruker Biflex III spectrometer equipped with a 337 nm N_2 laser in the positive linear mode and at a 19 kV accelerating voltage. α-Cyano-4-hydroxycinnamic acid was used as the matrix. Samples were prepared from a THF solution by mixing the matrix (20 mg/mL) and sample (10 mg/mL) in a ratio of 10:1. External mass calibration was performed using a standard peptide mixture.

Transmission electron microscopy (TEM) was performed on a JEM-100CX II TEM at an accelerating voltage of 100 kV. A drop of the micelle solution was deposited onto a carbon-coated copper grid and dried at room temperature. Then the grid was stained by a small drop of 2% phosphotungstic acid for 10 min before observation.

Dynamic light scattering (DLS) measurements were conducted at 25 °C on a Brookhaven BI-200SM apparatus with a BI-9000AT digital correlator and a He−Ne laser at 532 nm. Prior to the measurement, the sample solutions were filtered through nylon filters (13-HV, Millipore, 0.45 μm pore size). The data were analyzed by CONTIN algorithm, while the hydrodynamic radius

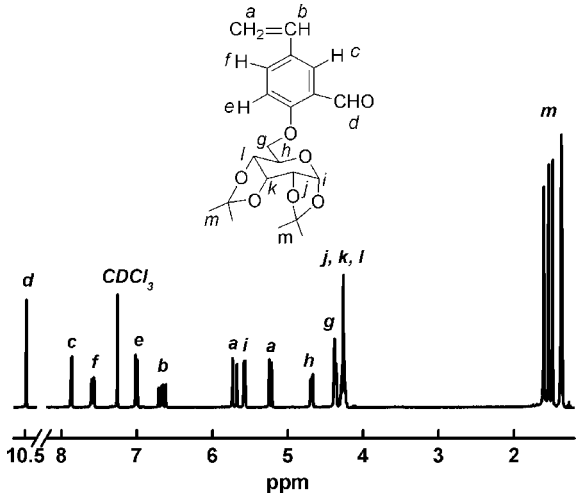

Figure 1. ^{1}H NMR spectra (300 MHz, $CDCl_3$, room temperature) of 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)-D-galactopyranose (IVDG).

(R_h) and size polydispersity of the micelles were obtained by a cumulant analysis of the experimental correlation function.

Results and Discussion

1. Monomer Synthesis and Its Conventional Radical Polymerization. The new glycomonomer IVDG carrying aldehyde functional groups was designed and prepared readily by the reaction of 1,2:3,4-di-*O*-isopropylidene-6-*O*-(*p*-toluenesulfonyl)-D-galactopyranose (ITDG) with 2-hydroxy-5-vinylbezaldehyde (Scheme 1), and its structure was confirmed via elemental analysis, MS, and NMR spectroscopy. The ^{1}H NMR spectrum exhibits the characteristic absorptions corresponding to vinyl (*a, b*), aldehyde (*d*), and acetal-protected sugar moiety (*g*−*m*); the other signals were assigned as shown in Figure 1.

Prior to the "living"/controlled radical polymerization, the conventional radical polymerization of the new monomer IVDG was investigated with AIBN in THF at 60 °C. A white and powdery polymer with high molecular weight (M_n = 132 100, M_w/M_n = 2.20, measured by GPC) was obtained at 68% conversion in 24 h ([IVDG] = 1.2 M, [AIBN] = 1.8 mM).

The thermal properties of the polymer were studied using TGA and DSC. The polymer exhibited good thermal stability until 320 °C (Figure 2) and had a relatively high glass transition temperature (T_g) at 130.6 °C (Figure 3) due to its bulky substituents.

2. RAFT Polymerization of IVDG with PEDB. *a. Polymerization Kinetics.* The RAFT process is one of the most robust and versatile techniques used to obtain control over the radical polymerization process because it is effective especially for a wide range of functional monomers and does not require stringent reaction conditions.[44] RAFT polymerization of IVDG was then attempted using AIBN as the initiator and PEDB as the RAFT agent at 60 °C in THF. PEDB was selected because of its versatility for a wide range of monomers such as styrenes,[45,46] acrylic acid,[47] and (meth)acrylates.[48] Note that THF was purified carefully via distillation and employed promptly in this study because of the possible effect of peroxide impurities in the solvent.[49]

To demonstrate the "living"/controlled nature of the RAFT polymerization, the polymerization kinetics was investigated. Figure 4 depicts the semilogarithmic kinetic plot for the polymerization. Figure 5 shows the corresponding plots of M_n and molecular weight distributions of the obtained polymers against the monomer conversions. The polymerization followed first-order kinetics up to about 85% conversion, indicating a

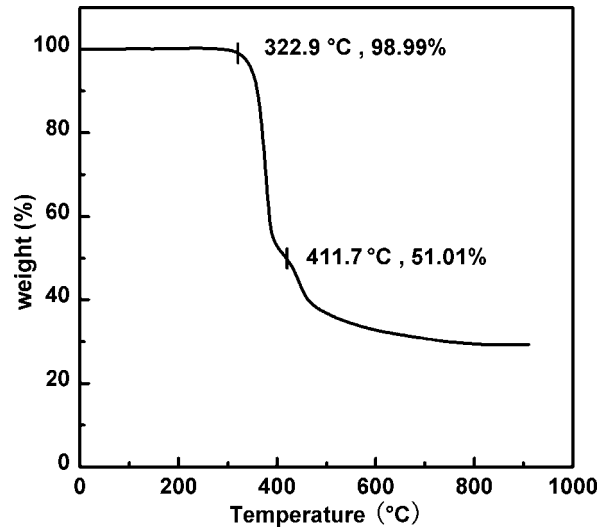

Figure 2. Thermogravimetric analysis thermogram of polyIVDG measured under nitrogen at a heating rate of 20 °C/min. PolyIVDG (M_n measured by gel permeation chromatography ~ 132 100, M_w/M_n ~ 2.20) was obtained with 2,2′-azobis(isobutyronitrile) (AIBN) at 60 °C in tetrahydrofuran for 24 h: [IVDG] = 1.2 M, [AIBN] = 1.8 mM. IVDG = 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)-D-galactopyranose; M_n and M_w are the number- and weight-average molecular weights, respectively.

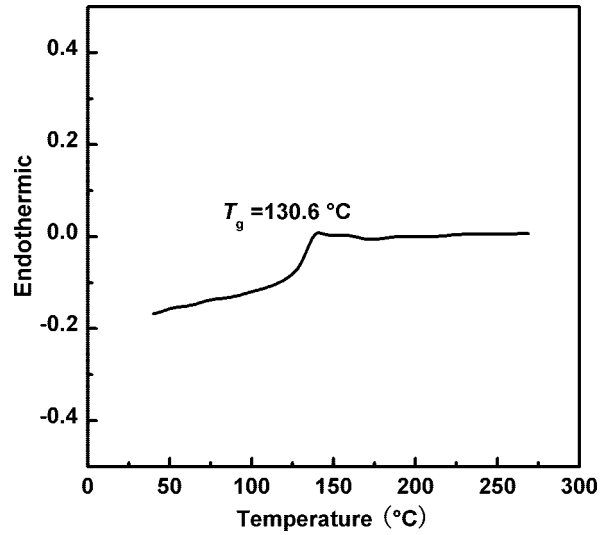

Figure 3. Differential scanning calorimetry analysis of polyIVDG measured under nitrogen at a heating rate of 10 °C/min. PolyIVDG (M_n measured by gel permeation chromatography ~ 132 100, M_w/M_n ~ 2.20) was obtained with 2,2′-azobis(isobutyronitrile) (AIBN) at 60 °C in tetrahydrofuran for 24 h: [IVDG] =1.2 M, [AIBN] = 1.8 mM. IVDG = 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)-D-galactopyranose; M_n and M_w are the number- and weight-average molecular weights, respectively.

constant concentration of propagating radicals during the polymerization. As normally evidenced for a "living"/controlled polymerization, M_n increased almost linearly with the monomer conversion, and the molecular weight distributions were narrow (M_w/M_n < 1.1) although they became slightly broader as the polymerization proceeded.

The M_n of the polymers measured by GPC based on standard polystyrene calibration in this study was systematically lower than the theoretical value (M_n = [IVDG]$_0$ × M_{IVDG} × conversion/[PEDB]$_0$ + M_{PEDB}; M_{IVDG} and M_{PEDB} are the molecular weights of IVDG and PEDB, respectively). This discrepancy may be mostly due to the different hydrodynamic volume between polyIVDG and polystyrene.

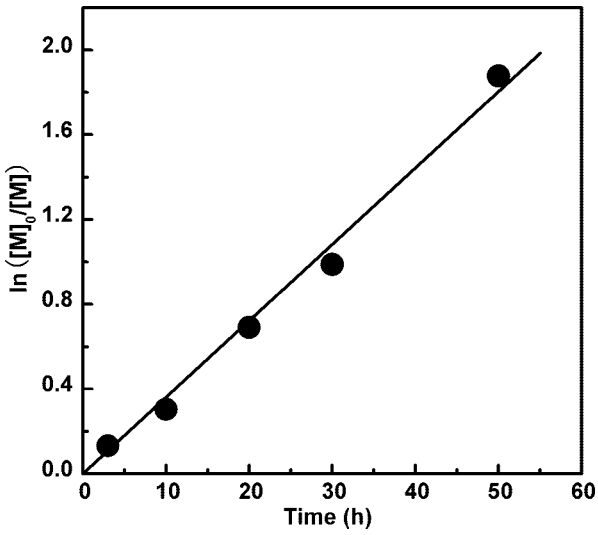

Figure 4. Semilogarithmic kinetic plot for the reversible addition−fragmentation chain transfer (RAFT) polymerization of IVDG with 2,2′-azobis(isobutyronitrile) (AIBN) as an initiator and 1-phenylethyl dithiobenzoate (PEDB) as a RAFT agent at 60 °C in tetrahydrofuran: [IVDG] = 0.8 M, [AIBN] = 3.75 mM, [PEDB] = 6 mM. IVDG = 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)-D-galactopyranose.

b. Polymer Analysis. The structure of the resulting polymer was analyzed by ^{1}H NMR spectroscopy. Figure 6 shows the ^{1}H NMR spectrum of a typical sample (M_n measured by GPC ~2040, M_w/M_n ~ 1.04) obtained with AIBN/PEDB in THF. The spectrum shows the characteristic signals for the main-chain repeating units of IVDG, i.e., the aldehyde protons (*b*), sugar residue protons (*c*−*i*), aromatic protons (*a*), and aliphatic protons (*j, k*). The ratio of the integration area of aldehyde protons vs aromatic protons in the polymer was 1:3, indicating the essential absence of side reactions on the aldehyde functionality of IVDG during the RAFT polymerization. In addition to these large absorptions, small signals due to the end groups appeared at 7.3–8.0 ppm. They are attributed to the aromatic protons (*m*) of PEDB fragment at the ω-chain end. Moreover, there appeared an absorption at 3.5 ppm, which corresponds to the end-standing methine proton (*l*) of IVDG unit capped with a PEDB fragment. Therefore, the polymer chain ends were capped with the RAFT agent fragments as expected according to the well-known mechanism of the RAFT process. Moreover, M_n of the polymer was estimated from the ^{1}H NMR peak intensity ratio of the aldehyde proton (*b*) to the end-standing methine proton (*l*) [M_n(NMR) = $M_{IVDG} \times b/l + M_{PEDB}$]. The M_n(NMR) value is 2710, which is close to the theoretical M_n (2860) based on the molar ratio of the consumed monomers to the RAFT agent.

The fine structure of polyIVDG obtained by the RAFT polymerization was further confirmed by MALDI-TOF-MS. As shown in Figure 7, there was a single main series of peaks, each almost separated by an interval of 390 Da, which corresponds to the molecular weight of the IVDG monomer. The absolute value of each peak was very close to the molecular weight expected for the polymer (depending on the degree of polymerization) with PEDB fragments at the α- and ω-chain ends that have a total mass of 258 Da. A minor series of peaks with a 153 Da lower mass were also observed, which suggested the loss of dithiobenzoate fragment at the ω-chain end during the MS analysis.[5]

3. Polymer Micelle Formation and Its Covalent Immobilization of BSA. *a. Deprotection of PolyIVDG.* Removal of the protective isopropylidene groups from the sugar residues in polyIVDG was carried out using 88% formic acid at room

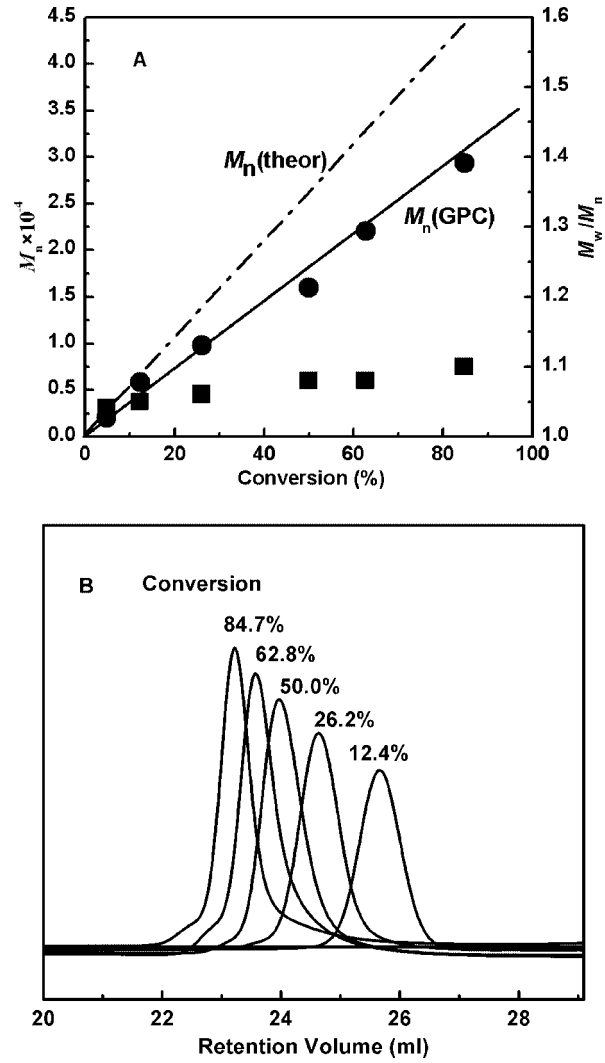

Figure 5. (A) M_n (●), M_w/M_n (■) and (B) molecular weight distribution curves of polyIVDG obtained with 2,2′-azobis(isobutyronitrile) (AIBN) as an initiator and 1-phenylethyl dithiobenzoate (PEDB) as a RAFT agent at 60 °C in tetrahydrofuran: [IVDG] = 0.8 M, [AIBN] = 3.75 mM, [PEDB] = 6 mM. IVDG: 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)-D-galactopyranose; M_n and M_w are the number- and weight-average molecular weights, respectively; M_n(theor) is the theoretical M_n, and M_n(GPC) is the M_n measured by gel permeation chromatography.

temperature. The deprotected polyIVDG with galactopyranose groups was finally obtained by dialysis against water and freeze-drying.[42] Figure 8 shows the ^{1}H NMR spectrum of a representative deprotected polymer. The signals of the isopropylidene protons (~1.2 ppm) completely disappeared after the deprotection, indicating the complete hydrolysis of the protecting acetal groups. As expected, the anomeric hydroxyl protons (*c*) of sugar moieties (6.1−7.0 ppm[50]) could not be observed separately because their signals were overlapped with those of the aromatic protons (*a*) of the main-chain repeating units. Importantly, the ratio of the integration area of the aldehyde protons (*b*) vs backbone aliphatic protons (*d*, *e*) in the polymer was calculated as 1:3, illustrating the essential absence of side reactions on the aldehyde functionality during the hydrolysis process.

b. Formation of Polymeric Micelles. After the hydrolysis of polyIVDG precursor, the resulting deprotected polymer with high water-soluble galactopyranose moieties is amphiphilic and expected to self-assemble into micelles in aqueous solution without recourse to any surfactant. Thus, preparation of the

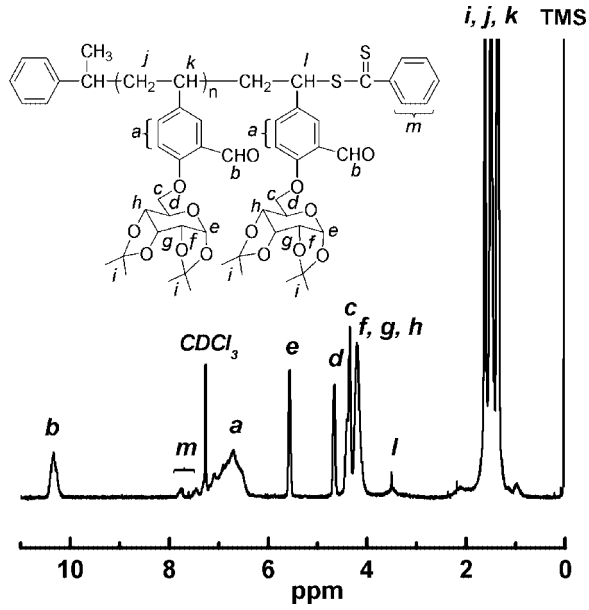

Figure 6. [1]H NMR (300 MHz, $CDCl_3$, room temperature) spectra of polyIVDG (M_n measured by gel permeation chromatography ~ 2040, M_w/M_n ~ 1.04) obtained with 2,2′-azobis(isobutyronitrile) (AIBN) as an initiator and 1-phenylethyl dithiobenzoate (PEDB) as a RAFT agent at 60 °C in tetrahydrofuran for 24 h: [IVDG] = 0.8 M, [AIBN] = 3.75 mM, [PEDB] = 6 mM. IVDG = 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)-D-galactopyranose; M_n and M_w are the number- and weight-average molecular weights, respectively.

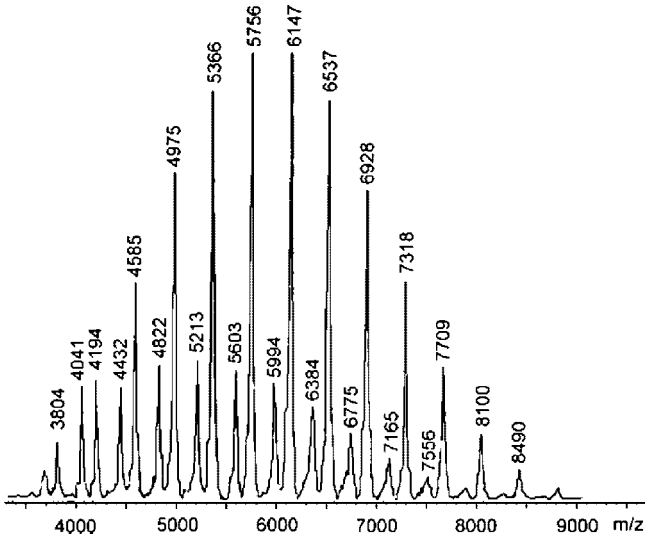

Figure 7. Matrix-assisted laser desorption−ionization time-of-flight mass spectrometry (MALDI-TOF-MS) spectrum of polymIVDG (M_n measured by gel permeation chromatography ~ 2040, M_w/M_n ~ 1.04) obtained with 2,2′-azobis(isobutyronitrile) (AIBN) as an initiator and 1-phenylethyl dithiobenzoate (PEDB) as a RAFT agent at 60 °C in tetrahydrofuran: [IVDG] = 0.8 M, [AIBN] = 3.75 mM, [PEDB] = 6 mM. IVDG = 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)-D-galactopyranose; M_n and M_w are the number- and weight-average molecular weights, respectively.

micelle was attempted by dissolving the deprotected glycopolymers in DMSO, followed by the addition of water to the polymer solutions. No precipitation was found after the addition of water; instead, the solution turned from completely colorless and transparent to slightly turbid, indicating the formation of micelles. After removal of the solvent DMSO by dialyzing, aqueous micellar solutions were finally obtained. The resulting aqueous micellar solutions are very stable, as demonstrated by the fact that they did not seem to change within about 6 months

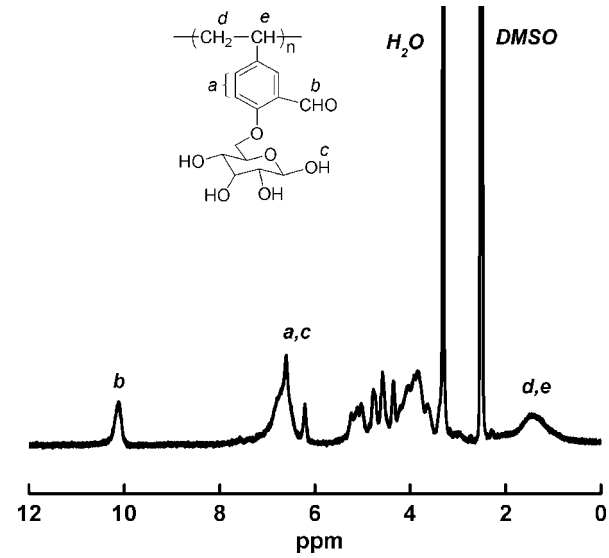

Figure 8. [1]H NMR (300 MHz, DMSO-d_6, room temperature) spectrum taken after the hydrolysis of polyIVDG (M_n measured by gel permeation chromatography ~ 13 000, M_w/M_n ~ 1.09) obtained with 2,2′-azobis(isobutyronitrile) (AIBN) as an initiator and 1-phenylethyl dithiobenzoate (PEDB) as a RAFT agent at 60 °C in tetrahydrofuran: [IVDG] = 0.8 M, [AIBN] = 3.75 mM, [PEDB] = 6 mM. IVDG = 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)-D-galactopyranose. M_n and M_w are the number- and weight-average molecular weights, respectively.

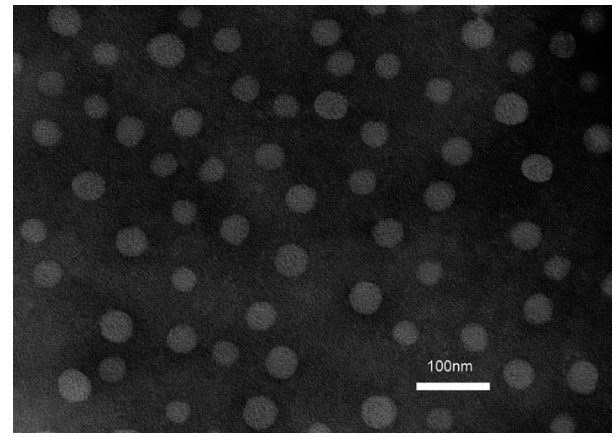

Figure 9. TEM micrograph of the micelles from the amphiphilic glycopolymer obtained by the hydrolysis of polyIVDG (M_n measured by gel permeation chromatography ~ 13 000, M_w/M_n ~ 1.09). IVDG = 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)-D-galactopyranose. M_n and M_w are the number- and weight-average molecular weights, respectively.

at ambient temperature. The micelles could be observed directly under TEM. Figure 9 shows the TEM image of a typical micelle sample (M_n of polyIVDG precursor ~13 000). The micrograph displayed discrete spherical particles with size in nanometer scale.

The hydrodynamic radius (R_h) and the size polydispersity of the micelles were determined by DLS in CONTIN algorithm. The amphiphilic glycopolymers, obtained by the hydrolysis of polyIVDG precursors with varying molecular weight in the range from 3×10^3 to 6×10^4, formed micelles with size in the 80−205 nm range. In all cases, the size distributions were monomodal, and the size polydispersity was typical of well-defined objects, i.e., lower than 0.1. As seen from Figure 10, the size of the micelles increased almost linearly with the molecular weight of polyIVDG precursor. Namely, the micelle size is readily adjustable by just changing the molecular weight

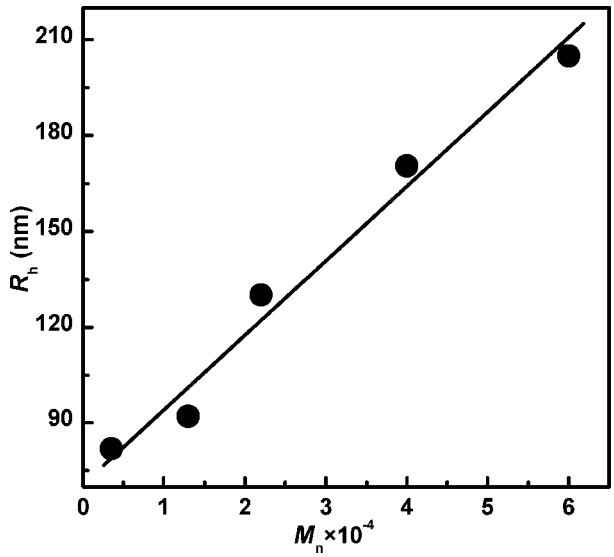

Figure 10. M_n dependence of R_h (measured by dynamic light scattering at scattering angle 90°) of the micelles from the amphiphilic glycopolymer obtained by the hydrolysis of polyIVDG. IVDG = 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)-D-galactopyranose. M_n = number-average molecular weights. R_h = hydrodynamic radius.

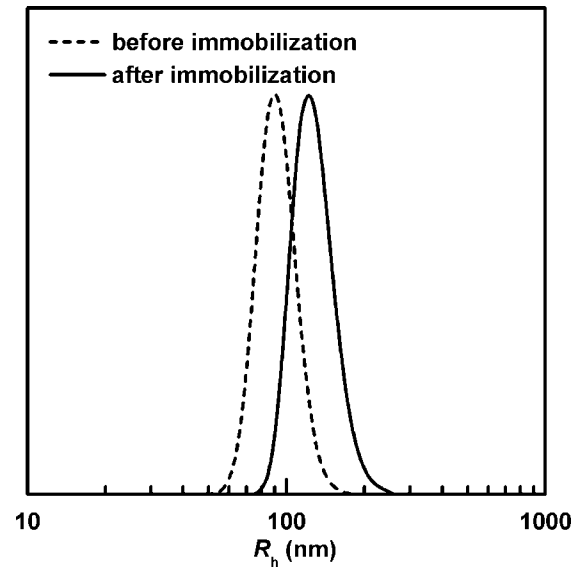

Figure 11. CONTIN size distributions (measured by dynamic light scattering at scattering angle 90°) of the nanospheres before and after the immobilization of bovine serum albumin. R_h = hydrodynamic radius.

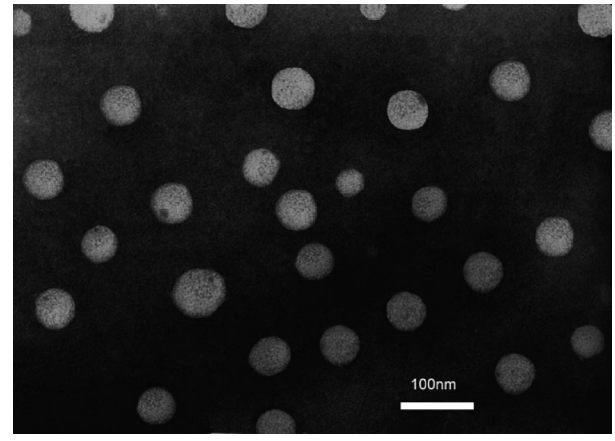

Figure 12. TEM micrograph of the bovine serum albumin (BSA)-attached nanospheres obtained by the immobilization of BSA onto the polymeric micelles (the same sample as used for Figure 9, M_n of polyIVDG precursor ~13 000).

of polyIVDG precursor, where the latter can be controlled directly via the RAFT living polymerization described above. Thus, well-defined micelles with controllable size are successfully prepared with a novel amphiphilic glycopolymers derived from polyIVDG precursors.

c. BSA Covalent Immobilization. One of the features of the polymeric micelles obtained above is that it bears multiple highly reactive aldehyde groups. Aldehyde groups can react under mild conditions with primary amino groups via the formation of Schiff base linkage, which provide a convenience for covalent immobilization of biomolecules, such as proteins, for applications in medicine and biotechnology.[6–9,51–53] Thus, the aldehyde-functionalized micelles were further conjugated with a model protein BSA, aiming at preparing a new type of bioactive polymeric nanospheres. The immobilization was performed by mixing the polymeric micelle solution (the same sample as used for Figure 9, M_n of polyIVDG precursor ~13 000) with aqueous BSA solution for 8 h under stirring at 25 °C.[53] The particles with attached BSA were separated by centrifugation (18 000 rpm for 1 h at 4 °C), and the supernatants were analyzed for the immobilized BSA concentration by the standard Bradford protein assay method.[43] The resulting BSA-immobilized particles could be redispersed readily in water to form a colloid solution. Figure 11 shows the CONTIN size distributions measured by DLS before and after the immobilization of BSA. The clear shift of the nanoparticle toward bigger size direction was observed after the immobilization, indicative of the formation of polymeric micelle−protein (BSA) bioconjugates. The BSA-immobilized nanoparticles could also be observed directly under TEM. As in Figure 12, the micrograph displayed nanospheres with increased size compared to the case before the immobilization (cf. Figure 9). The protein concentration analysis results showed that aldehyde-functionalized micelles had a high ability to immobilize protein BSA, and the immobilization capacity of the present sample was as high as 201 mg BSA/g micelle particles.

Conclusion

The RAFT polymerization of a new aldehyde-functionalized glycomonomer, IVDG, was successfully carried out using AIBN as the initiator and PEDB as the RAFT agent at 60 °C in THF. The polymerization showed typical "living"/controlled free radical polymerization behaviors. The hydrolysis of polyIVDG resulted in a novel amphiphilic polymers which could self-assemble into well-defined multiple aldehyde-bearing polymeric micelles in aqueous solution without recourse to any surfactant. The micelle size was readily adjustable by the molecular weight of polyIVDG precursor. Protein-bioconjugated nanoparticles were also successfully prepared by the immobilization of BSA onto the aldehyde-functionalized micelles.

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