Synthesis of Thermosensitive Polymers Containing Sugar Branches

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ABSTRACT: Copolymers of 6-*O*-vinyladipoyl-D-glucose (VAG) and *N*-isopropyl acrylamide (NIPAm) were synthesized by radical polymerization. The number-average molecular weights of the copolymers were $3 \times 10^4 \approx 6 \times 10^4$. The observed segment composition of copolymers at the feed molar ratio (VAG 25/NIPAm 75) was VAG 10/NIPAm 90. The polymerization rate of the VAG monomer was slower than that of the NIPAm monomer. The lower critical-solution temperature of copolymers measured with a light-scattering photometer and a differential scanning calorimeter increased with increasing VAG segment composition. The increase in transition temperature was accompanied by a decrease in transition heat. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 80: 384–387, 2001

Key words: polymerizable sugar ester; vinyl adipoyl sugar; *N*-isopropylacrylamide; thermosensitive polymer; copolymerization

INTRODUCTION

Synthetic polymers containing sugar branched have attracted considerable interest. Several researchers have developed polymers that have sugar branches such as polystyrene containing *N*-acetyl-β-lactosamine moieties,[1] glucosyl oxyethylmethacrylate,[2] vinyl sucrose derivatives,[3] sucrose-based polyacrylate,[4] and others.[5,6] We reported the enzyme-catalyzed synthesis of a polymerizable sugar ester, 6-*O*-vinyladipoyl-D-glucose (VAG), and its polymerization to give polymer containing glucose branches.[7] Our strategy did not merely glucose but also mannose, galactose, arabinose, and adenosine.[8–10] It is important to investigate the function of a sugar-branch polymer on its development as an intelligent material. Kobayashi et al. observed that polymers containing *N*-acetyl-β-lactosamine interact with the wheat-germ agglutinin lectin.[11] Hatanaka et al. reported that copolymers containing uridine inhibit galactocyl transferase.[5] In a previous work,[12] we reported the superoxide generation activity of poly(6-*O*-vinyladipoyl-D-glucose) measured by nitro blue tetrazolium reduction. These reactions are important in biological systems such as those for the inactivation of viruses and the cleavage of DNA.[13]

Poly(*N*-isopropyl acrylamide) (PNIPAm) has a lower critical solution temperature (LCST), about 31°C[14], in aqueous solution. The thermosensitive behavior of PNIPAm has been extensively investigated.[15–20] In recent studies PNIPAm and N-isopropyl acrylamide (NIPAm) copolymers have been used in immunoassays,[15] drug delivery,[16-18] separation processes,[19] and immobilization of enzymes.[20] Monji and Hoffman successfully used poly(*N*-isopropyl acrylamide-*co*-*N*-acryloxysuccinimide) in an-

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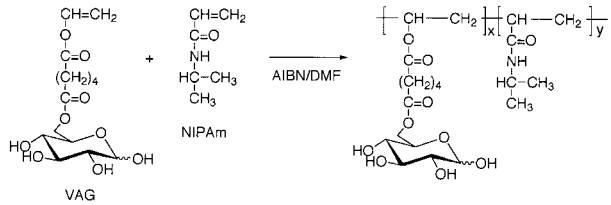

Scheme 1

tibody immobilization studies.[15] Biological applications usually involve the chemical modification of PNIPAm, which can be achieved by copolymerization of NIPAm with functional comonomers.

To develop a thermosensitive sugar polymer possessing a recognize function of biopolymers such as protein, nucleic acid, polysaccharide, sugar chain, virus, microorganism, cell and so on, copolymers of VAG and NIPAm were prepared by radical polymerization. There are few reports about copolymers with acrylamide derivatives of sugar and NIPAm.[21,22] Zhou et al. reported the synthesis and properties of hydrogel composed of the acrylamide derivatives of lactose and NIPAm.[21] Kim et al. successfully used poly (*N*-isopropyl acrylamide-*co*-acrylamide-2-deoxy-D-glucose) in conjugation with α-chymotrypsin.[22] Hence, to our knowledge, no report have been made on the introduction of a sugar vinyl ester to PNIPAm. The main focus of this article is to report on our study ascertaining the LCST behavior of copolymers by using a light-scattering photometer and a differential scanning calorimeter (DSC).

EXPERIMENTAL

Materials

VAG was prepared by enzymatic synthesis according to our previous published method.[7] NIPAm was obtained from Kohjin Co., Ltd. (Japan). 1,1′-Azobisisobutyronitrile (AIBN) was purchased from Nacalai Tesque, Inc. (Japan). All solvents used were analytical grade and were redistilled after being dried.

Polymerization Procedure

Copolymerization of VAG and NIPAm was carried out as follows: in a 10-mL sealed polymerization tube a mixture containing VAG (0.127 g), NIPAm(0.125 g), AIBN (5 mg), and dimethyl formamide (DMF) (0.5 mg) was maintained at 60°C for 24 h under vacuum. The resulting product was precipitated in diethyl ether and dried under reduced pressure at 40°C.

Analytical Methods

The average molecular weight of the polymer was determined by size exclusion chromatography (SEC) with a refractive index detector (Tosoh HLC-8020). A combined column, TSKα − M + TSKα − 4000, was used with a mobile phase of DMF containing 10 mmol of LiBr at a flow rate of 0.6 mL/min. Polystyrene of $500 \leq M_W \leq 8.42 \times 10^6$, Tosoh was used as a molecular-weight standard. The copolymer composition was estimated by elemental analysis (PerkinElmer 2400 II).

Cloud-Point Measurement

The cloud point of 0.5% distilled water of the copolymer sample solution was determined by measuring the increase of optical density at 547 nm using a light-scattering photometer (Shimadzu UV-160A) equipped with a thermoregulated block bath. The temperature scanning rate was 0.2 °C/min.

Transition-Point Measurement

Measurement of the transition point of 5% distilled water solution of the copolymer samples

Table I Copolymerization of VAG and NIPAm

Feed ratio VAG:NIPAm	Yield (%)	M_n ($\times 10^4$)	M_w ($\times 10^5$)	M_w/M_n	Composition VAG:NIPAm
100 : 0	65	3.34	1.49	4.5	100 : 0
25 : 75	50	5.91	1.78	3.2	10 : 90
15 : 85	32	5.49	2.04	3.7	7 : 93
5 : 95	22	3.8	1.58	4.1	4 : 96
0 : 100	62	3.34	1.49	4.4	0 : 100

was carried out with a DSC (Seiko Instruments DSC-220C). The scanning rate was 0.5°C/min.

RESULTS AND DISCUSSION

As shown in Scheme 1, the random copolymers of VAG and NIPAm were prepared with a conventional azo-initiator (AIBN) in DMF at 60°C. The copolymerization of VAG with NIPAm is summarized in Table I. The segment composition ratios (*x/y*) of copolymers were estimated from the nitrogen content of the NIPAm moiety. The M_n of these copolymers was $3 \times 10^4 \approx 6 \times 10^4$ and the M_w/M_n values were 3≈5. The observed segment composition of copolymer at the feed molar ratio (VAG 25/NIPAm 75) was VAG10/NIPAm 90. In all cases, the polymerization rate of VAG monomer was slower than that of NIPAm monomer.

Figure 1 shows the cloud point of copolymers made from VAG and NIPAm. The cloud-point temperature of copolymers is about 32–38°C. The cloud-point temperature increased with increasing VAG segment composition.

Figure 2 shows the transition temperature of copolymers made from VAG and NIPAm. As expected, each transition temperature agreed closely with the cloud point of the corresponding copolymer. We found that the copolymers from VAG and NIPAm have a clear LCST behavior. The copolymer transition temperature increases with increasing VAG segment composition. The increase in transition temperature is accompanied by a decrease in transition heat. So far, few have reported that NIPAm copolymer with a highly hydrophilic sugar had clear LCST behavior. Further, without the use of sugar, several studies have shown that LCST behavior depends

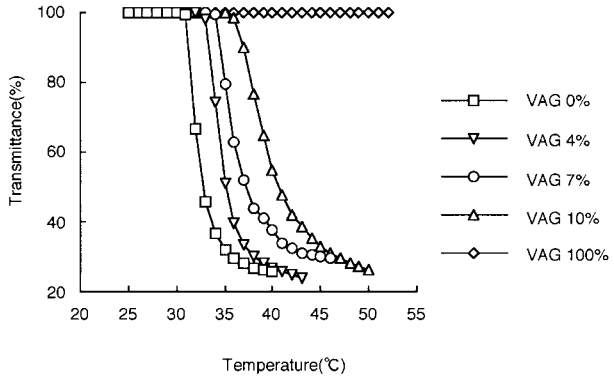

Figure 1 Turbidity of 0.2 wt % solution of copolymers.

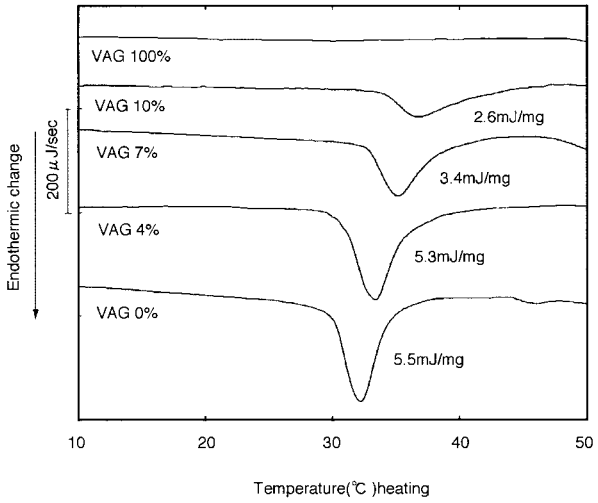

Figure 2 DSC thermograms of 5 wt % solution of copolymers.

on the critical hydrophilic–hydrophobic balance of the polymer side groups.[23–30] Feil et al. reported that the introduction of a hydrophilic acrylamide group to PNIPAm shifted the transition temperature high and decreased the transition heat.[30] Our copolymers can recognize, take, and release various biopolymers in a body temperature comparable to PNIPAm without a protein denaturation. Its polymers are useful in bioseparation and drug delivery systems having recognizable functions toward biopolymers. The vinyl adipoyl sugar ester in our copolymers, would interact with a biopolymer stronger than an acrylamide derivative of sugar because of the long-chain spacer of the vinyl adipoyl ester, which decreases the effect of steric hindrance on the polymer main chain. The substitution of a glucose moiety for another sugar, nucleoside, and sugar derivative spread the applicability of this copolymer.

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