Reversible Formation of Nanoparticles by Thermoresponsive Dehydroalanine Polymer: Poly(*N*-isobutyryldehydroalanine methyl ester)

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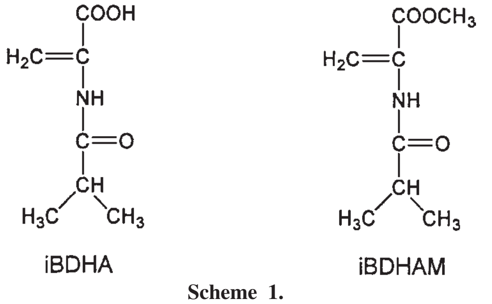
Two kinds of dehydroalanine monomers, *N*-isobutyryl dehydroalanine (iBDHA) and its methyl ester (iBDHAM), were synthesized and their homopolymers were prepared by free radical polymerization. While poly(iBDHA) did not show any phase transition in water, poly(iBDHAM) exhibited the lower critical solution temperature (LCST) at 19 °C to form colloidal dispersion of nanoparticles with 60 nm diameter above the LCST. This nanoparticle formation was thermoreversible.

Thermoresponsive polymer is an intelligent polymeric material which shows macromolecular phase transition in response to external temperature change. Some amphiphilic polymers[1] exhibit heat-induced phase separation in aqueous solutions, the temperature of which is referred to as the lower critical solution temperature (LCST). The best known example is poly(*N*-isopropylacrylamide), poly(NIPAAm), which shows the LCST at 32 °C.[2] Various derivatives of the poly(NIPAAm) and other types of amphiphilic polymers have been synthesized in the purpose of controlling the LCST and the phase transition behavior. Bifunctional polymers bearing two kinds of functional groups at every monomer unit have advantage in the molecular design to control hydrophilic-hydrophobic balance in the macromolecular structure, specific interaction between the polymer and the solvent, structurization of water around hydrophobic groups, and intra- and intermolecular hydrogen bonding; these properties and interactions play a dominant role in the phase transition. *N*-Acyl derivatives of dehydroalanine (DHA) are promising candidates for this purpose because they readily polymerize to yield high molecular weight polymers.[3] This high polymerizability is attributed to enhanced stabilization of propagating radicals by sinergetic effect of an electron-withdrawing and an electron-donating group based on the concept of captodative stabilization. In the course of our study on the synthesis and polymerization of DHA monomers,[4] we have found that homopolymers of *N*-isobutyryldehydroalanine (iBDHA) and its methyl ester (iBDHAM) exhibit the LCST in aqueous (electrolyte) solutions. We report here the synthesis of these homopolymers and their phase transition behavior.



Scheme 1.

Synthesis of iBDHA and iBDHAM: Isobutyramide (0.29 mol, 25 g) and pyruvic acid (0.58 mol, 51 g) were refluxed in benzene (200 mL) for 8 h with removing water. The solvent was evaporated on a rotary evaporator and the residue was cooled in a freezer for 12 h. The precipitate was collected by vacuum filtration and recrystallized from acetone, producing iBDHA as white crystals[5] (m.p. 158–162 °C) in 14% yield. The purified iBDHA (0.020 mol, 3.1 g) and potassium carbonate (0.044 mol, 6.1 g) were refluxed in acetone (250 mL) with stirring, and iodomethane (0.040 mol, 5.7 g) was added dropwise for 10 min. The reaction mixture was refluxed for 6 h with stirring, and additional iodomethane (0.020 mol, 2.8 g) was added at the middle of the refluxing period. The reaction mixture was allowed to cool at the room temperature and the precipitate was removed by filtration. The filtrate was evaporated on a rotary evaporator and the residue was extracted with hexane. The extract was washed twice with water and dried with magnesium sulfate. By removing the solvent under reduced pressure at room temperature, iBDHAM of 99.9% purity (determined by gas chromatography) was obtained as colorless liquid[6] in 67% yield.

Polymerization of iBDHA and iBDHAM was conducted in dimethyl sulfoxide (DMSO) solutions at a monomer concentration of $1.0\,\mathrm{mol\,dm^{-3}}$ using ammonium peroxodisulfate ($5.0 \times 10^{-3}\,\mathrm{mol\,dm^{-3}}$) as an initiator at 30 °C for 30 h in degassed and sealed ampoules. The crude polymer solutions were diluted by methanol, and then poly(iBDHA) was precipitated from acetone and poly(iBDHAM) from diethyl ether. The polymer yields for poly(iBDHA) and poly(iBDHAM) were 59% and 73%, respectively. The weight average molecular weights, M_w, of the poly(iBDHA) and the poly(iBDHAM), determined by the static light scattering (SLS)measurement,[7] were 5.0×10^4 and 3.0×10^5, respectively. Both the homopolymers were soluble in water and various polar solvents such as alcohols and acetone. Poly(iBDHAM) was also soluble in some nonpolar solvents such as benzene and toluene at concentrations higher than $1.0\,\mathrm{g\,L^{-1}}$, indicating that the poly(iBDHAM) has highly amphiphilic property.

Poly(iBDHA) did not show any phase transition in water at any polymer concentration, c, over the temperature range 10 to 80 °C. Heat-induced phase separation was only observed at extreme conditions such as very high ionic strength or very low pH as shown in Figure 1. Since poly(*N*-vinylisobutyramide), a caboxy derivative of which is the poly(iBDHA), exhibits the LCST at 39 °C in water,[8] it is suggested that strong hydration of the carboxyl groups and electorostatic repulsion between carboxylate anions even at acidic solutions prevent the hydrophobic association of isopropyl groups at the other side chains.

Esterification of these carboxyl groups caused a drastic change in the phase transition behavior: Poly(iBDHAM)

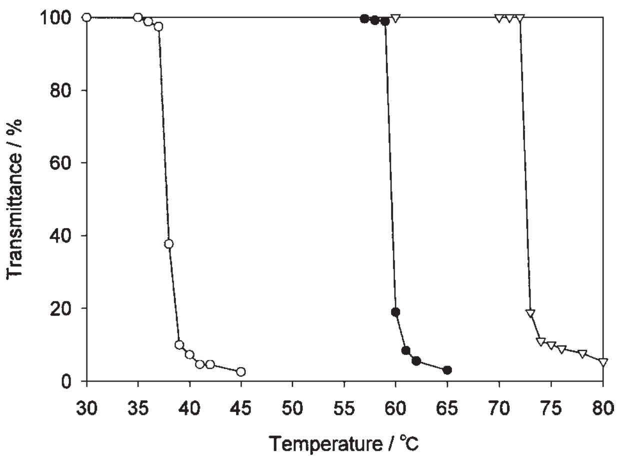


Figure 1. Temperature dependence of light transmittance at 500 nm for poly(iBDHA) in electrolyte solutions ($c = 2.0\,\mathrm{g\,L^{-1}}$): (○) 2.6 mol dm^{-3} NaCl aq, (●) 1.7 mol dm^{-3} NaCl aq, (▽) KCl-HCl buffer solution at pH = 1.0, heating up.

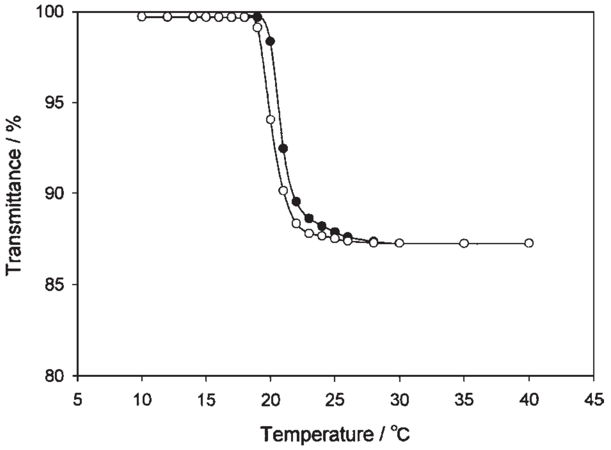


Figure 2. Temperature dependence of light transmittance at 500 nm for poly(iBDHAM) in water ($c = 2.0\,\mathrm{g\,L^{-1}}$): (●) heating up and (○) cooling down.

exhibited the LCST at 19 °C in water (Figure 2). Although the phase transition occurred within a narrow temperature range, the change in the light transmittance was very small. In the visual observation, the cold clear solution changed into slightly bluish cloudy when the temperature was raised above the LCST, and it returned to clear when the temperature was lowered below the LSCT.

In order to characterize the reversible formation of the colloidal dispersion, we have conducted the dynamic light scattering (DLS) measurement at various temperatures.[7] At 15 °C, the scattering intensity was extremely low and the analyzed hydrodynamic diameter was 20–30 nm, suggesting that the poly(iBDHAM) does not associate in dilute solutions, similarly to the poly(NIPAAm) in cold water.[2,9] As the temperature was raised above the LCST, the scattering intensity increased by 10^3 times. Figure 3 shows the particle size distribution obtained by histogram (NNLS) analysis of the DLS data at 25 °C. The average diameter 59.9 nm and standard deviation ±2.5 nm indicate the formation of monodisperse nanoparticles. The cumulant analysis gave almost the same value (59.4 nm). The property of thermoreversible formation of nanoparticles reveals the potential application of this polymer to various fields, similarly to the other types of thermosensitive nanoparticle forming polymers.[9–12]

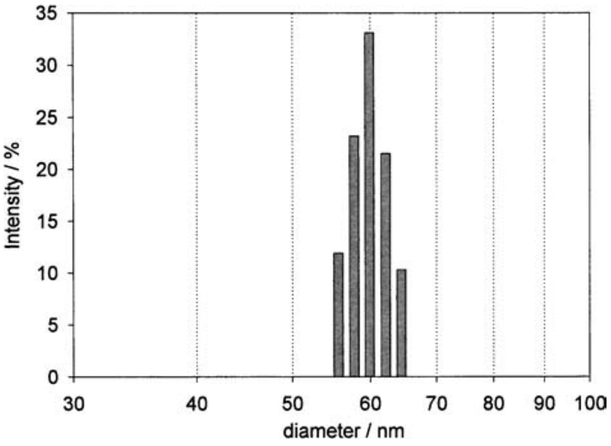


Figure 3. Particle size distribution of poly(iBDHAM) in water ($c = 1.0\,\mathrm{g\,L^{-1}}$) at 25 °C determined by DLS analysis.

While the nanoparticle dispersion of the poly(iBDHAM) was stable in water (for at least three months), it immediately coagulated into the precipitate even in 0.05 mol dm^{-3} NaCl aq. These results indicate that the surface charge on the nanoparticles prevents their aggregation. This surface charge is probably derived from the sulfate anions introduced at the end of the polymer chain from the initiator. The surface charge or the electrical double layer can be stabilized by an appropriate orientation of dipole moments arising from the polar groups in the bifunctional dehydroalanine polymer.

References and Notes

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5 Data for iBDHA. Elemental analysis: Calcd for $C_7H_{11}NO_3$: C, 53.49; H, 7.05; N, 8.91%. Found: C, 53.23; H, 6.93; N, 8.90%. ^{1}H NMR ($(CD_3)_2SO$): δ 1.04 [d, 6H, $\text{-CH(C}\underline{\text{H}}_3)_2$], 2.72 [sept, 1H, $\text{-C}\underline{\text{H}}(\text{CH}_3)_2$], 5.68 [s, 1H, CH =], 6.26 [s, 1H, CH =], 8.93 [broad s, 1H, -NH] ppm. MS: m/z (relative intensity, %) 157 (M, 100), 158 ($M + 1$, 9.6).

6 Data for iBDHAM. Elemental analysis: Calcd for $C_8H_{13}NO_3$: C, 56.12; H, 7.65; N, 8.18%. Found: C, 56.08; H, 7.37; N, 8.39%. ^{1}H NMR ($(CD_3)_2SO$): δ 1.08 [d, 6H, $\text{-CH(C}\underline{\text{H}}_3)_2$], 2.73 [sept, 1H, $\text{-C}\underline{\text{H}}(\text{CH}_3)_2$], 3.78 [s, 3H, -COOCH_3], 5.70 [s, 1H, CH =], 6.24 [s, 1H, CH =], 9.08 [broad s, 1H, -NH] ppm. MS: m/z (relative intensity, %) 171 (M, 100), 172 ($M + 1$, 10).

7 DLS and SLS measurements were conducted on a light scattering spectrophotometer, DLS-7000 (Photal), equipped with a He-Ne laser (633 nm, 10 mW) and an Ar laser (488 nm, 75 mW).

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