Preparation and Physical Properties of Thermoresponsive Biodegradable Poly(asparagine) Derivatives

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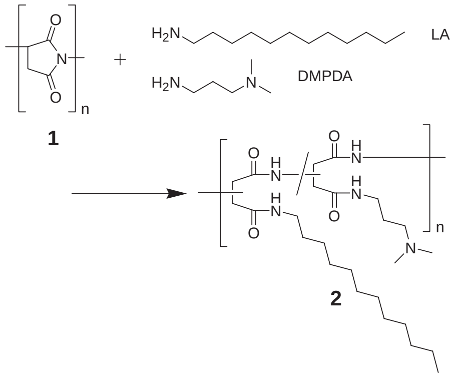
New biodegradable and thermoresponsive poly(asparagine) derivatives have been developed. They have a LCST and showed a sol–gel–sol phase transition. The LCST and a sol–gel–sol phase transition temperature could be controlled by the composition ratio of hydrophilic and hydrophobic groups of the polymer side chain.

Recently, there has been great interest in polymers exhibiting the phase transition that is readily reversible with thermal stimulus. Among them, poly(*N*-isopropylacrylamide) (PNIPAM) is currently the most extensively studied thermoresponsive polymer.[1] PNIPAM, NIPAM-containing copolymers, and PNIPAM-based crosslinked hydrogels have been widely applied to controlled drug delivery, biomedical materials, fillers of column chromatography, gene-transfection agents, immobilized biocatalysts, and others.[2] However, non-biodegradability of PNIPAM may restrict its applications in the biomedical field. It was reported that aqueous solutions of block copolymers of biodegradable poly(lactic acid)–poly(ethylene glycol) showed the sol–gel transition.[3] Amphiphilic block copolymers showing thermogelation upon heating were reported. Typical examples are poly(ethylene glycol)–*block*–poly(propylene glycol) and poly(2-(2-ethoxy)ethoxyethyl vinyl ether)–*block*–poly(2-methoxyethyl vinyl ether).[4]

Poly(amino acid)s are biodegradable and biocompatible materials, useful for various bio-related industries.[5] Poly(α/β-aspartic acid), which is synthesized by thermal polymerization of aspartic acid, followed by alkaline hydrolysis, is expected to be an alternative to non-biodegradable poly(acrylic acid) widely used in various industrial fields. Recently, it was reported that the poly(aspartic acid) derivatives formed the micelle in the aqueous solution, and new thermo-responsive polymers based on biodegradable poly(amino acid)s, poly(N-substituted α/β-asparagine)s, which showed a clear lower critical solution temperature (LCST) in water, have been developed.[6] However, these thermoresponsive poly(asparagine)s did not show a sol–gel–sol phase transition in water.



Scheme 1.

This study deals with new amphiphilic poly(N-substituted α/β-asparagine)s showing sharp thermoresponsive properties. By precise control of the introduction of the hydrophilic 3-dimethylaminopropyl group and the hydrophobic dodecyl group into the side chain of poly(α/β-asparagine)s (Scheme 1), not only demonstrated a phase separation in a dilute aqueous solution but also a physical gelation in a concentrated aqueous solution.

For the synthesis of thermoresponsive poly(asparagine)s (**2**), poly(succinimide) (**1**) with M_w and M_w/M_n of 9.8×10^4 and 1.45, respectively, was used as a precursor. The reaction of **1** with a mixture of dodecylamine (LA) and *N*,*N*-dimethyl-1,3-propanediamine (DMPDA) was carried out in *N*,*N*-dimethylformamide at 70 °C for 6 h, in which the succinimide group of **1** was quantitatively reacted to form a N-substituted asparagine unit. The content of LA and DMPDA in the polymer was determined by ^{1}H NMR spectroscopy.[7] In this study, five polymer samples with different LA content were synthesized. Table 1 summarizes their LA content and physical properties. All of these polymers were soluble in water at low temperature. On the other hand, the polymer with the LA content higher than 58% was not soluble in water, even at 5 °C.

Figure 1 shows the temperature dependence of the viscosity of 10 wt % aqueous solutions of polymers C, D, and E on the heating process in the temperature range from 5 °C to 80 °C.[8] Polymer E showed a significant increase in viscosity, and the maximum viscosity of polymer E was observed at 24 °C. Around this temperature, the solution apparently turned into a gel due to the very high viscosity. Above this temperature, the viscosity rapidly decreased. Similar behavior was observed in polymers C and D. As the LA content in polymer C, D, and E increased, the temperature of the maximum viscosity (T_{mv}) decreased and the maximum viscosity (η_{max}) increased. On the other hand, only

Table 1. Composition and thermoresponsive properties of **2**

Polymer	Content of LA[a]/%	Viscosity[b]		Gelation[b]		LCST[c]
		η_{max}[d]/Pa·s	T_{mv}[e]/°C	T_{sg}[f]/°C	T_{gs}[g]/°C	T_{ps}/°C
A	27	—	—	—	—	78
B	37	3.70×10^{-1}	62	—	—	56
C	42	1.41×10^{1}	44	—	—	44
D	48	9.95×10^{1}	32	18	39	28
E	52	2.33×10^{2}	24	10	35	—

[a]Determined by ^{1}H NMR, [b]Measured with the 10 wt % polymer aqueous solutions, [c]Measured with the 1 wt % polymer aqueous solutions, [d]Maximum viscosity, [e]Maximum viscosity temperature, [f]Transition temperature from sol to gel phase, [g]Transition temperature from gel to sol phase.

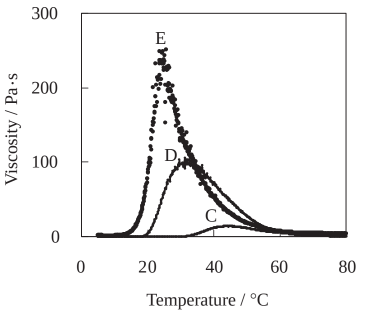


Figure 1. Temperature dependence of apparent viscosity for 10 wt % polymer aqueous solutions (polymers C–E).

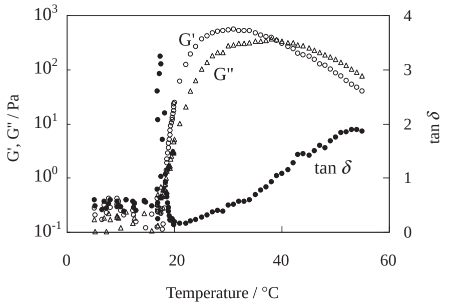


Figure 2. Temperature dependence of storage modulus (G′), loss modulus (G″) and tan δ for 10 wt % polymer D aqueous solution.

a very small change in the viscosity was seen in polymer B, and the viscosity of polymer A scarcely changed in the temperatures measured.

In order to elucidate the present thermogelation behavior, the dynamic viscoelasticity of the polymer solution was measured. Figure 2 depicted traces of storage modulus (G′), loss modulus (G″), and tanδ of 10 wt % solution of polymer D. Below 18 °C, G″ was larger than G′ (G″ > G′). This confirmed that the polymer solution behaves like sol below this temperature. At 18 °C, the G′ value suddenly increased to the same as G″ (G″ = G′, tan δ = 1), indicating that the phase transition from sol-like elasticity-state to gel-like viscosity-state (sol–gel phase transition) took place. In the range from 18 °C to 39 °C, the gel phase (G″ < G′) was observed. Above 39 °C, the order was reversed again (G″ > G′), at which the gel phase returned to the sol phase (gel–sol phase transition). These phenomenona clearly showed a sol–gel–sol phase transition of polymer D with thermal stimulus. In the case of polymer E, the sol–gel phase transition occurred at 10 °C and the gel–sol phase transition occurred at 35 °C. For polymer C, G′ was smaller than G″ (G″ > G′) in the region of all the temperatures examined, although a viscosity increase was observed. The same relation between G′ and G″ was also seen in polymers A and B. These data suggested that the gel phase was not observed in polymer A, B, and C.

Moreover, these polymer solutions of the dilute concentration exhibited the thermally responsive phase separation. The temperature dependence of the transmittance is shown in Figure 3. Polymers A–D exhibited a sharp phase separation. Temperature at 50% of transmittance on the heating process is defined as the LCST (T_{ps}). As the content of the hydrophobic substitutent (LA) increased, T_{ps} decreased. By changing the compositeon ratio of LA and DMPDA in **2**, T_{ps} could be accurately controlled over a wide range from room temperature to around 80 °C.

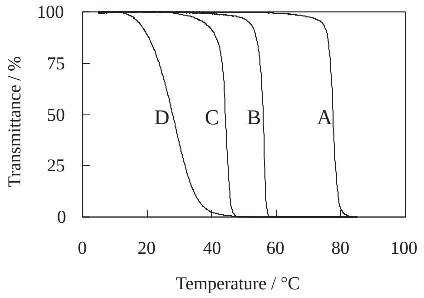


Figure 3. Temperature dependence of light beam transmittance on heating process; 1 wt % solution, wavelength = 500 nm, heating rate = 1 °C/min.

In conclusion, we have developed new thermoresponsive and biodegradable poly(asparagine) derivatives (**2**) which show not only the LCST but also a sol–gel–sol phase transition. These thermoresponsive properties could be precisely controlled by changing the composition of the side chain in **2**. The present unique sol–gel–sol phase transition and the LCST of **2** has great potential for various applications. Further works concerning applications of the present thermoresponsive poly(amino acid)s and the elucidation of the mechanism of a sol–gel–sol phase transition are ongoing in our laboratory.

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References and Notes

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7 The composition of the side chain in **2** was determined by the integrated ratio of peaks of methyl protons of the dodecyl group (3H, t, 0.89 ppm) and central methylene protons derived from DMPDA (2H, b, 1.71 ppm) in the ^{1}H NMR spectrum of **2**.

8 The viscoelastic properties of polymers were measured by a stress-control-type rheometer with a parallel plate with a diameter of 40 mm at a heating ratio of 1.0 °C/min at a frequently of 1.0 Hz.

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