New Biodegradable and Thermoresponsive Polymers Based on Amphiphilic Poly(asparagine) Derivatives

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Summary: New biodegradable and thermoresponsive polymers based on amphiphilic poly(asparagine) derivatives have been developed. They showed a sol-gel-sol phase transition. In particular, not only a phase transition behavior but also a lower critical solution temperature (LCST) was observed in the polymers with *N,N*-dimethylaminopropyl groups as the hydrophilic side chains. The LCST and a sol-gel-sol phase transition temperature could be precisely controlled by changing the composition ratio and kind of the hydrophobic and the hydrophilic groups of the side chains in the polymers. These polymers are expected to be applicable for controlled release of drugs, tissue engineering, and others.

Keywords: biodegradable; hydrogels; poly(amino acid)s; stimuli-sensitive polymers

Introduction

There has been great interest in polymers exhibiting the phase transition that is readily reversible with thermal stimulus. Thermoresponsive polymers have been widely investigated for various applications such as controlled drug delivery, biomedical materials, fillers of column chromatography, gene-transfection agents, immobilized biocatalysts, and others.[1–8] Poly (*N*-isopropylacrylamide) (PNIPAM) is currently the most extensively studied thermoresponsive polymer. PNIPAM exhibits a rapid and reversible hydration-dehydration change in response to small temperature cycles around its LCST.[9–11] In addition to PNIPAM and NIPAM-containing copolymers, various thermoresponsive polymers, typically poly(vinyl methyl ether), poly(2-isopropyl-2-oxazoline), poly(*N*-vinylalkylamide)s, and poly(phosphazene)s, have been developed.[12–15] However, non-biodegradability of these polymers may restrict their applications in the biomedical field.

Polypeptides and related artificial poly(amino acid)s have become important materials because of their specific properties of biodegradability, biocompatibility, and others.[16–18] 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 a micelle in an aqueous solution, and new thermoresponsive polymers based on biodegradable poly(amino acid)s, poly(*N*-substituted α/β-asparagine)s, which had a LCST in water, have been developed.[19–22] However, these thermoresponsive poly(*N*-substituted α/β-asparagine)s did not show a sol-gel-sol phase transition in water.

This study deals with new biodegradable and thermoresponsive amphiphilic poly(*N*- substituted α/β-asparagine)s showing sol-gel-sol phase transition behaviors. A concentrated aqueous solution of the polymers with the hydroxyl and the methoxy groups in the terminal moieties of the

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hydrophilic side chains showed a sol-gel-sol phase transition. In addition, not only a physical gelation in a concentrated aqueous solution but also a phase separation in a dilute aqueous solution was observed in the polymers with the tertiary amine in the terminal moieties of the hydrophilic side chains. By precise control of the introduction of the hydrophilic and the hydrophobic groups into the side chains of the polymers, the sol-gel (T_{sg}) and the gel-sol (T_{gs}) phase transition temperatures and the LCST can be easily regulated.

Experimental Part

General Procedure of Preparation of Thermoresponsive Polymers

A typical polymer preparation was as follows (sample **2**). Poly(succinimide) (PSI) (**1**) (9.7 g, 0.1 mol) was dissolved in *N,N*-dimethylformamide (DMF) (34 g) in a separable flask equipped with a mechanical stirrer and a thermometer. A mixture of dodecylamine (LA) (6.5 g, 0.035 mol) and 2-methoxyethylamine (MOE) (4.9 g, 0.065 mol) was added. The reaction mixture was kept at 70 °C for 6 hours. The resulting solution was poured into a large amount of acetonitrile. The formed precipitates were collected by filtration and dried at 60 °C for 24 hours (19.4 g, 92% yield). All other polymers were obtained in the same manner.

Measurements

The dynamic viscosity and viscoelasticity of the polymer solutions and gels were measured using a stress-control-type rheometer (Viscoanalyzer Var-50/100, Reologica Instrument, AB). A parallel plate with a diameter of 40 mm was employed. The dynamic viscoelastic measurements were carried out at varying temperatures with a frequency of 1.0 Hz at a heating rate of 2.0 °C/min. The temperature was controlled within 0.1 °C by a Peltier element. The LCST of an aqueous polymer solution was determined from a light beam transmittance at 500 nm through a 1.0 cm quartz cell, monitored at a rate of 1.0 °C/min on heating process scans between 5 and 85 °C. The transmittance was recorded on a Shimadzu UV-2500PC UV/vis spectrometer equipped with a Peltier-type thermostatic cell holder PC-TEC controller.

Results and Discussion

The new biodegradable and thermoresponsive amphiphilic poly(*N*-substituted α/β-asparagine)s were prepared by the reaction of PSI (**1**) with a combination of the hydrophobic and the hydrophilic amines at 70 °C in DMF (Figure 1). The quantitative introduction of the amine moiety was achieved by the addition of a mixture of the hydrophobic and the hydrophilic amines for PSI (**1**). The feed ratio of each amine and thermoresponsive physical properties of the prepared samples are summarized in Table 1.

Samples **2**, **3a-c**, **4**, **5**, and **6a–d** are the thermoresponsive polymers with the methoxyethyl groups, the hydroxypropyl groups, the hydroxybutyl groups, the hydroxypentyl groups, and the *N,N*-dimethylaminopropyl groups as the hydrophilic side chains, respectively.

Ten weight percent (wt%) aqueous polymer solution of **2** behaved as a liquid below room temperature. On the other hand, it turned into a transparent hydrogel near body temperature. This hydrogel was transformed to a free-flowing solution again with further heating. The free-flowing solution at high temperatures returned through the gel phase to a solution at low temperatures by cooling down to below room temperature. These thermoresponsive phase transition behaviors were observed by the repetition of the heating and cooling process. To elucidate the characteristic behavior of the thermoresponsive sol-gel and gel-sol phase transitions of the polymer, the dynamic viscoelastic properties of an aqueous solution of **2** were investigated. Figure 2 shows the temperature dependence of the dynamic viscosity, the storage modulus (G'), the loss

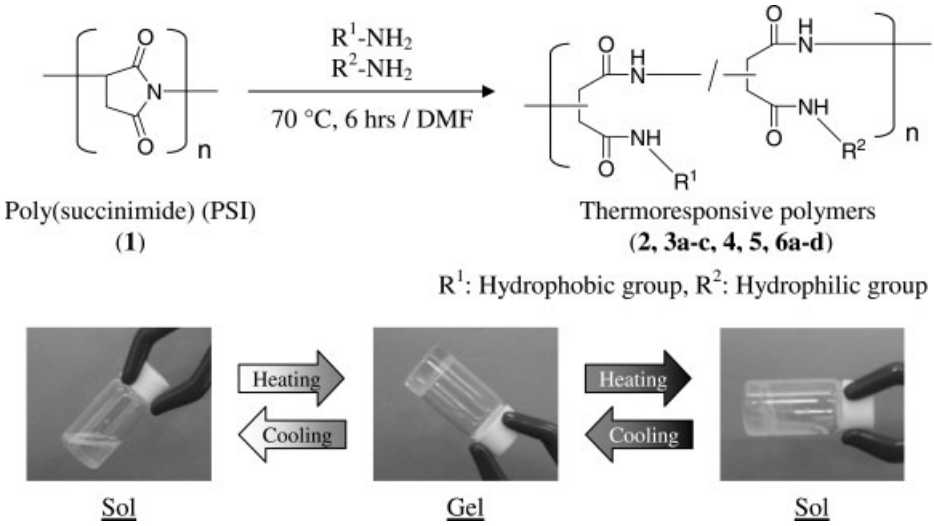

Figure 1.
Preparation and a sol-gel-sol phase transition of thermoresponsive poly(*N*-substituted α/β-asparagine)s.

modulus (G''), and tan δ of a 10 wt% aqueous solution of **2**. Below room temperature, G') was smaller than G'' (tan $\delta > 1$). At about 20 °C, both values suddenly increased, and G') became larger than G''; this indicated the formation of a gel. With further heating, G' again became smaller than G''. A similar behavior was observed in the temperature dependence of the dynamic viscosity. These data clearly show that this polymer has a sol-gel-sol phase transition in aqueous media with an external heat stimulus.

The effects of the polymer concentration of **2** on these phase transition temperatures were examined (Figure 3). The T_{sg} and the T_{gs} were defined as $G' = G''$ (tan $\delta = 1$). In the region of tan $\delta \leq 1$, the solution behaved as a gel. The T_{sg}, the T_{gs}, and the temperature range of the gel phase strongly depended on the polymer concentration. Below a concentration of 5 wt%, the

Table 1.
Composition ratio and physical properties of thermoresponsive polymers.

Sample	R^1	R^2	$R^1/R^{2a)}$	Gelation [b]		LCST [c]
				T_{sg}/°C [d]	T_{sg}/°C [e]	T_{ps}/°C
2	$-(CH_2)_{11}CH_3$	$-(CH_2)_2$-OCH_3	35/65	19	65	-
3a	$-(CH_2)_{11}CH_3$	$-(CH_2)_3$-OH	50/50	19	49	-
3b	$-(CH_2)_{11}CH_3$	$-(CH_2)_3$-OH	45/55	32	51	-
3c	$-(CH_2)_{11}CH_3$	$-(CH_2)_3$-OH	40/60	45	54	-
4	$-(CH_2)_{11}CH_3$	$-(CH_2)_4$-OH	40/60	25	72	-
5	$-(CH_2)_{11}CH_3$	$-(CH_2)_5$-OH	40/60	22	38	-
6a	$-(CH_2)_{11}CH_3$	$-(CH_2)_3$-$N(CH_3)_2$	45/55	18	39	28
6b	$-(CH_2)_{11}CH_3$	$-(CH_2)_3$-$N(CH_3)_2$	40/60	–	–	44
6c	$-(CH_2)_{11}CH_3$	$-(CH_2)_3$-$N(CH_3)_2$	35/65	–	–	56
6d	$-(CH_2)_{11}CH_3$	$-(CH_2)_3$-$N(CH_3)_2$	30/70	–	–	78

[a] Feed ratio of the hydrophobic and the hydrophilic amines;
[b] Measured with the 10 wt% polymer aqueous solutions;
[c] Measured with the 1 wt% polymer aqueous solutions;
[d] Transition temperature from sol to gel;
[e] Transition temperature from gel to sol.

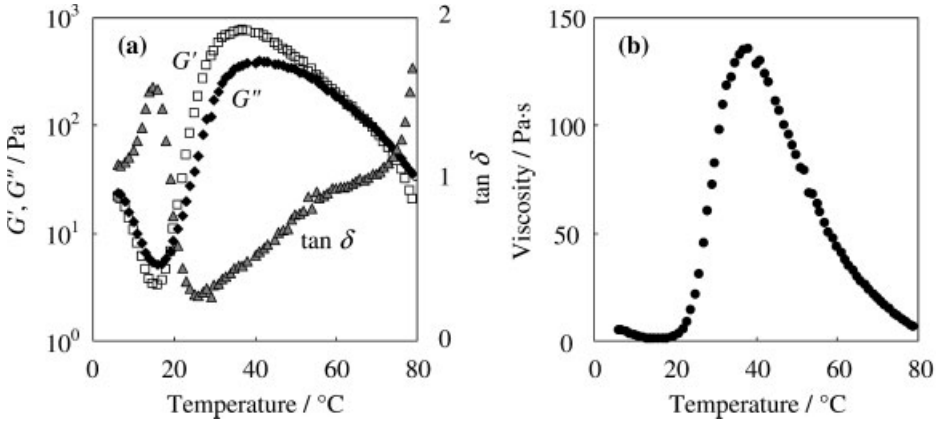

Figure 2.
Temperature dependence of the dynamic viscoelasticity and viscosity of a 10 wt% aqueous polymer solution (sample **2**): (a) G', G'', and tan δ and (b) dynamic viscosity. The rheometry measurements were carried out at frequency of 1.0 Hz at a heating rate of 2.0 °C/min.

sol-gel-sol phase transition was not detected. At the higher concentration of the polymer, the gel phase was found in the wider temperature range.

As noted above, the polymer with the methoxy groups in the terminal moieties of the hydrophilic side chains showed a sol-gel-sol phase transition with thermal stimulus. In addition, the polymers, which were allowed to react with 3-amino-1-propanol (AP) instead of MOE, also showed a sol-gel-sol phase transition. Figure 4 depicts the effects of the composition of the side chains on these phase transition temperatures of 10 wt% aqueous polymer solutions. The T_{sg} and the T_{gs} increased linearly as a function of the introduction ratio of the hydrophilic AP

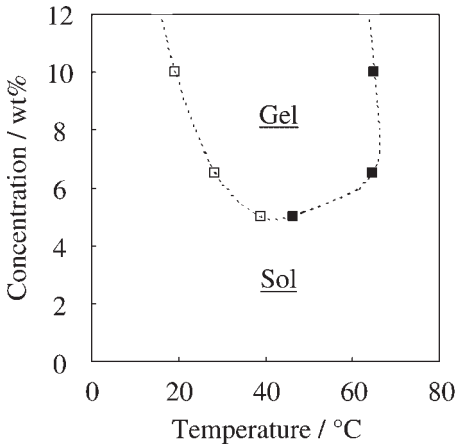

Figure 3.
Effect of the polymer concentration on the temperature range of the gel phase of thermoresponsive polymer (sample **2**): (□) phase transition temperatures from sol to gel (T_{sg}) and (■) phase transition temperatures from gel to sol (T_{gs}).

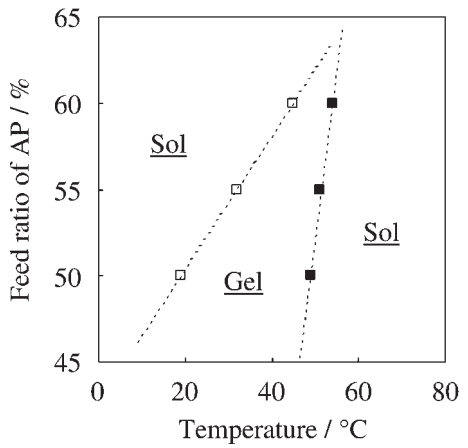

Figure 4.
Effect of the composition on the temperature range of the gel phase of 10 wt% polymer aqueous solutions (samples **3a–c**): (□) phase transition temperatures from sol to gel (T_{sg}) and (■) phase transition temperatures from gel to sol (T_{gs}).

unit in **3a–c** (correlation coefficient = 0.99). No phase transition was observed in the polymer containing the AP unit over 65% (data not shown). The T_{sg} strongly depended on the introduction ratio of the hydrophilic AP unit. On the other hand, the T_{gs} was less dependent on the composition of the polymer. The gel phase was found in the wider temperature range with an increase in hydrophobicity of the polymer. These phase transition temperatures and the temperature range of the gel phase could be controlled by not only the polymer concentration but also the composition ratio of the side chains in the polymer.

Moreover, these phase transition temperatures could be controlled by mixing a different kind of polymer aqueous solution. The temperature dependence of the dynamic viscosity of the individual and mixed polymer solutions is shown in Figure 5. The dynamic viscosity of a mixed aqueous solution of the same amount of the polymer with the hydroxybutyl group (**4**) and the hydroxypentyl group (**5**) was intermediate between those of individual polymer aqueous solutions.

Not only a sol-gel-sol phase transition but also a phase separation was observed in

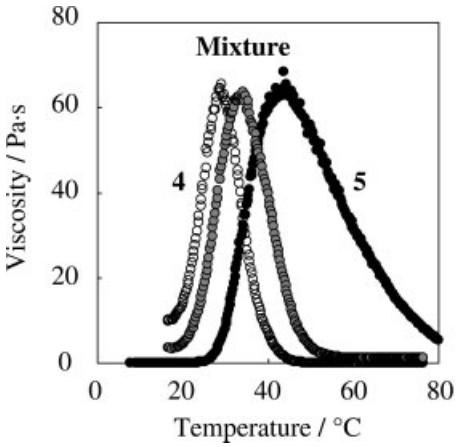

Figure 5.
Temperature dependence of the dynamic viscosity of thermoresponsive polymer: ○ = 10 wt% aqueous solution of **4**, ● = 10 wt% aqueous solution of **5**, ● = mixture of the same amount of ○ and ●. The rheometry measurements were carried out at frequency of 1.0 Hz at a heating rate of 2.0 °C/min.

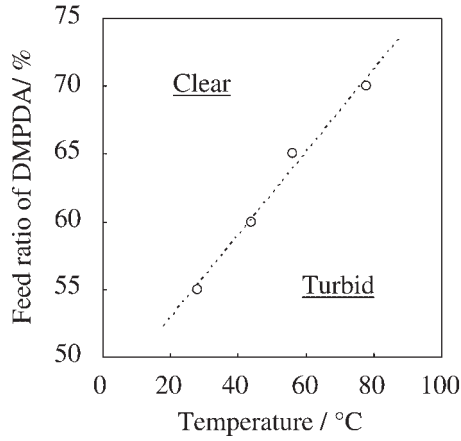

Figure 6.
Relationship between composition ratio of DMPDA in **6a–d** and the LCST. The LCST was defined as 50% transmittance. The measurements were performed at 500 nm at a heating rate of 1.0 °C/min.

the polymers that PSI (**1**) reacted with a mixture of LA and *N,N*-dimethyl-1,3-propanediamine (DMPDA). Figure 6 shows the temperature dependence of a light beam transmittance of 1 wt% aqueous solutions of **6a–d** at 500 nm on the heating process. For all the samples, the turbidity change took place sharply. In the cooling process, similar behavior was observed (data not shown). In this study, the LCST was defined as 50% transmittance of the polymer solution during the heating process. The LCST was in the wide range from room temperature to the approximate boiling point of water and linearly increased as a function of content of DMPDA in the polymer. These data indicated that the LCST could be also controlled by the composition ratio of LA and DMPDA units in the polymer.

Conclusions

New biodegradable and thermoresponsive polymers based on amphiphilic poly(*N*-substituted α/β-asparagine)s were developed. An aqueous solution of the amphiphilic poly(*N*-substituted α/β-asparagine)s exhibited a sol-gel-sol phase transition with

thermal stimulus. Not only a phase transition but also a phase separation was observed in the polymers that DMPDA was used as a hydrophilic amine. The T_{sg}, the T_{gs}, and the LCST strongly depended on the concentration of the polymer, the composition ratio of the side chain, the mixed ratio of the polymer aqueous solution, and the structure of the side chain in the polymer. These thermoresponsive physical properties could be easily regulated by precise control of the introduction of the hydrophilic and the hydrophobic groups into the side chains of the polymers.

These polymer aqueous solutions formed a hydrogel without any additives, and these materials are expected to be applicable for controlled release of drugs, tissue engineering, and others. Further studies on our polymers based on biodegradable poly(amino acid)s are under way in our laboratories.

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