

# A Versatile Biodegradable Polymer with a Thermo-Reversible/Irreversible Transition

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**ABSTRACT** A versatile biodegradable thermoresponsive polymer was developed. The polymer has succinimide and isopropylasparamide segments and exhibits a phase transition with thermoreversibility that can be controlled by changing the polymer composition. With fewer succinimide units, the polymer exhibits the type of thermo-reversible phase transition that is characteristic of poly(*N*-isopropylacrylamide) (PNIPAAm). The polymer with a higher proportion of succinimide units exhibits a thermo-irreversible phase transition, resulting in the formation of nanospheres that are stable below the transition temperature. The stable nanospheres are generated by dehydration and subsequent conformational stabilization through an interaction between imide rings. This thermoirreversible phase transition in water provides a simple, oil-free preparation of biodegradable nanospheres.

**KEYWORDS:** thermoresponsive polymer • irreversible phase transition • poly(aspartic acid) • biodegradable nanosphere • poly(succinimide)

Stimuli-responsive polymers have many potential applications because their properties change dramatically in response to external stimuli such as temperature, pH, and electric field (1–4). Thermoresponsive polymers whose water solubility changes abruptly in response to temperature have been extensively investigated for use in practical applications such as drug delivery vehicles, bioseparation reagents, and surface modifiers, as well as for academic interest to elucidate mechanisms of heat denaturation of proteins (5–7). Attention has mostly been paid to thermoresponsive polymers with lower critical solution temperatures (LCST) in water (8–11). These polymers are water-soluble below the LCST because of their hydrated extended chain conformation, and lose their water solubility above the LCST because of their nonhydrated collapsed chain conformation (12). Although polymer materials are often required to be biodegradable to be useful in fields such as medicine, biology, and environmental science, conventional thermoresponsive polymers such as poly(*N*-isopropylacrylamide) (PNIPAAm) and its copolymers are not degradable. Here, we report a versatile biodegradable thermoresponsive polymer with regulatable thermoreversibility that can be controlled by changing its composition.

Poly(aspartic acid) synthesized by acid-catalyzed polycondensation of *L*-aspartic acid followed by alkaline hydrolysis is known to be biodegradable and water-soluble (13, 14). Aminolysis of an intermediate product, poly(succinimide) (PSI), with nucleophilic amino compounds is used to form various poly(asparamide)s with functional groups in side chains (15–19). To synthesize thermoresponsive poly(asparamide), we used isopropylamine, which gave a PNIPAAm-

like side chain structure, because the thermoresponsivity of PNIPAAm is ascribed to its *N*-isopropylamide side chain structure. Poly[ $\alpha,\beta$ -(*DL*-aspartate isopropylamide)-*co*-(succinimide)] (designated IPA-PSI) was obtained (Scheme 1) by aminolysis of PSI with isopropylamine in dimethylformamide: the details of the synthesis are described in the Supporting Information. Because poly(aspartic acid)s with dodecyl groups in the side chains have showed biodegradability (16), poly(aspartate) was expected to be a biodegradable polymer. IPA-PSI was gradually converted into poly(aspartate) with thermoresponsive side chains by alkali hydrolysis; therefore IPA-PSI was also expected to be biodegradable.

The mole fraction of isopropylasparamide units in the polymer (hereafter referred to as the degree of substitution) was varied from 30 to 76 mol %. Complete aminolysis of succinimide with isopropylamine did not occur even at the highest mole ratio of isopropylamine to the succinimide unit of PSI in the feed. This might be due to the steric hindrance of the branched alkyl group of isopropylamine because the aminolysis reaction of PSI with propylamine, a nucleophile with a straight alkyl chain, proceeded almost to completion at the same mole ratio of propylamine to the succinimide unit of PSI. Aminolysis of one hydrophobic succinimide unit with one isopropylamine molecule produces one hydrophobic isopropyl group and two hydrophilic amide bonds, thus increasing the hydrophilicity of the polymer. Consequently, the degree of substitution (DS) has a significant effect on the water solubility of the synthesized IPA-PSI. The effect of DS on water solubility is illustrated in Figure 1a. PSI and IPA-PSI with DS < 30 mol % were consistently insoluble in ultra pure water (ultrafiltered with a Milli-Q water purification system) in the temperature range 0–100 °C, and their cloud points were not determined. IPA-PSI with DS > 37 mol %, on the other hand, dissolved freely in ultra pure water. The thermo-responsivity of water-soluble IPA-PSI was of two

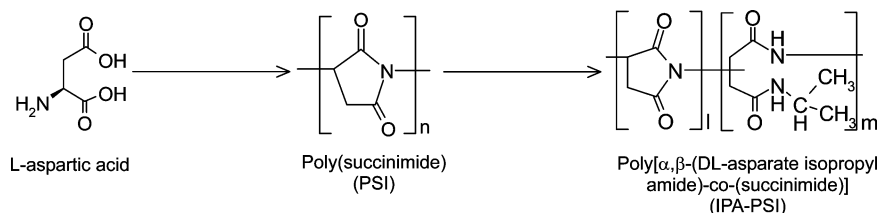
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## Scheme 1. Synthesis of IPA-PSI



types, corresponding to reversible and irreversible phase transitions, according to the magnitude of DS (Figure 1a).

Aqueous solutions of IPA-PSI with DS > 37 mol % became turbid after being heated to above their cloud point, due to dehydration of the thermoresponsive isopropylamide units. A reversible phase transition was observed for aqueous solutions of IPA-PSIs with DS in the range 62–76 mol %, which were transparent after cooling, as for a conventional thermo-responsive polymer (Figure 1b). By contrast, aqueous solutions of IPA-PSIs with DS in the range 37–56 mol % remained turbid even after cooling (Figure 1b), and the colloid dispersions remained stable for more than one week

even after cooling to 0 °C. The temperature dependences of transmittance for 1 wt % aqueous solutions of IPA-PSIs with DS 76 and 37 mol % are shown in Figure 1c together with reference curves for PNIPAAm for comparison. The method used to obtain the transmittance curves is given in the Supporting Information. It is apparent from this Figure that the transmittance of the aqueous solution of IPA-PSI with DS = 76 mol % changes more gradually than that of PNIPAAm, and the heating curve does not coincide with the cooling curve (as for PNIPAAm). The difference between the heating and cooling curves became increasingly pronounced with decreasing DS, implying that the phase transition of

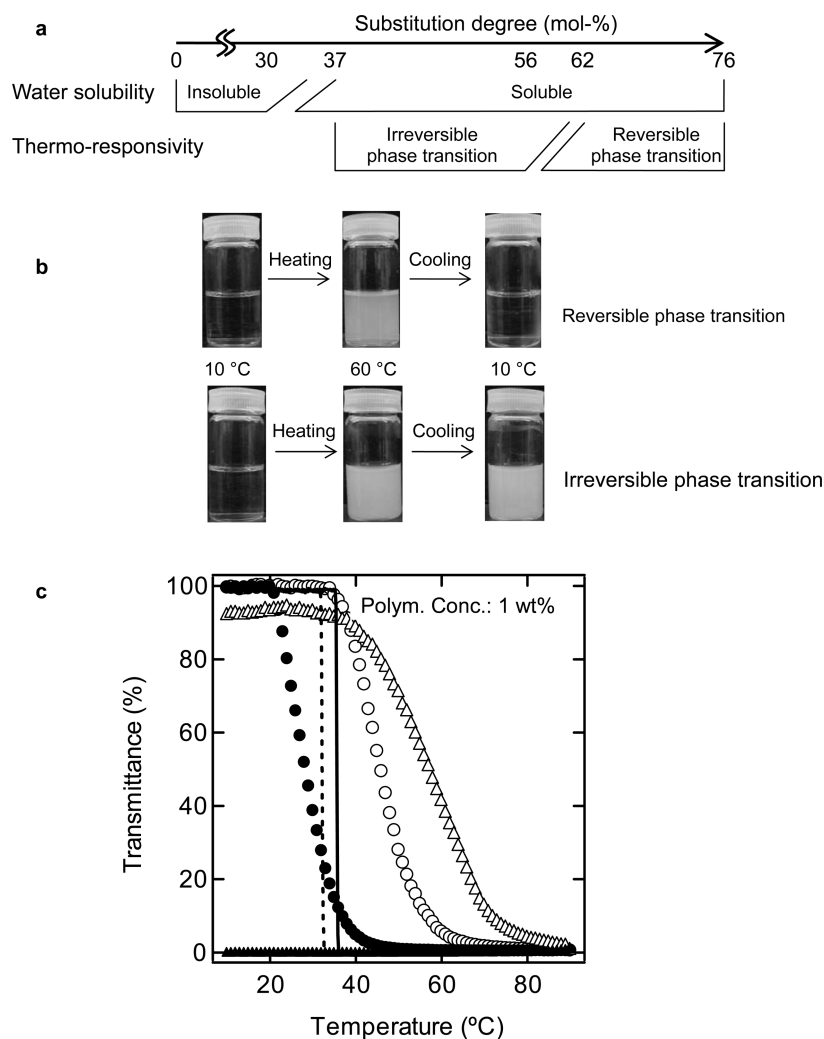
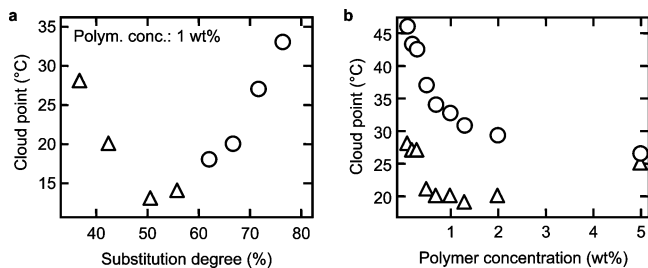


FIGURE 1. Thermoresponsiveness of aqueous solutions of IPA-PSI with various degrees of substitution (DS). (a) Effect of DS on water solubility and thermo-responsivity. (b) Change in appearance of aqueous IPA-PSI with DS = 76 (upper) and DS = 37 mol % (lower). (c) Transmittance of 1 wt % aqueous IPA-PSI solution as a function of temperature. Open triangles, heating curve for IPA-PSI with DS = 37 mol %; filled triangles, cooling curve for IPA-PSI with DS = 37 mol %; open circles, heating curve for IPA-PSI with DS = 76 mol %; filled circles, cooling curve for IPA-PSI with DS = 76 mol %; solid line, heating curve for PNIPAAm; dotted line, cooling curve for PNIPAAm.

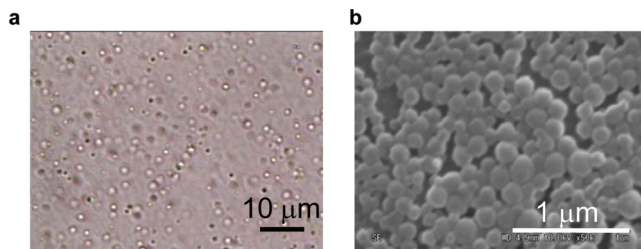


**FIGURE 2.** Dependence of the cloud point of aqueous IPA-PSI on DS and polymer concentration. (a) Effect of DS on cloud point of 1 wt % aqueous IPA-PSI solution. Triangles, cloud point of IPA-PSI showing irreversible phase transition; circles, cloud point of IPA-PSI showing reversible phase transition. (b) Effect of polymer concentration on cloud point of aqueous IPA-PSI solution. Triangles, DS = 43 mol %; circles, DS = 76 mol %.

IPA-PSI gradually becomes irreversible as DS decreases. The phase transition of IPA-PSI with DS < 60 mol % is irreversible, indicating that the succinimide segments in the main chain significantly affect the reversibility of the phase transition. Moreover, the sharpness of the phase transition decreased as DS decreased. This tendency is attributed to a decrease in the proportion of thermo-responsive isopropylamide units. A similar tendency has been reported for PNIPAAm copolymers with a functional group; the phase transition of PNIPAAm copolymer exhibits a more gradual transition than that of PNIPAAm homopolymer (20, 21).

The effect of DS on the cloud point of 1 wt % aqueous IPA-PSI solution is shown in Figure 2. The cloud point was strongly affected by DS and decreased as DS increased up to 50 mol %. Above that threshold, the cloud point increased as DS increased. It is reasonable to consider this peculiar tendency in two regions of DS. For IPA-PSI with DS > 50 mol %, the variation in the cloud point can be attributed to the hydrophilicity of IPA-PSI. Hydrophilic polymers form strong hydrogen bonds between the amido groups and water molecules, and require more energy to become dehydrated than do hydrophobic polymers, which implies that they have a higher cloud point. This tendency has been reported for copolymers of PNIPAAm and poly(ethylene oxide) (22–24).

For IPA-PSIs with DS < 50 mol %, the variation in the cloud point may be ascribed to increase of the number of intermolecular interaction sites. Furthermore, it seems that reducing the rigidity of the polymer backbone also accelerates intermolecular aggregation. It seems that a five-membered succinimide ring gives more rigidity compared with an aspartate unit because the glass transition temperature of the polymer is increased by decreasing DS of IPA-PSI (see Supporting Information). Consequently, for IPA-PSI with more succinimide rings to collapse, it must overcome a significant energy barrier, resulting in a higher cloud point. In addition, cloud points generally depend on the polymer concentration because aggregation is based on intermolecular interaction: it is known that PNIPAAm shows intramolecular aggregation triggered by dehydration during heating, referred to as a coil–globule transition (25). Figure 2 shows the decrease in the cloud points of IPA-PSI solutions with increasing polymer concentration.

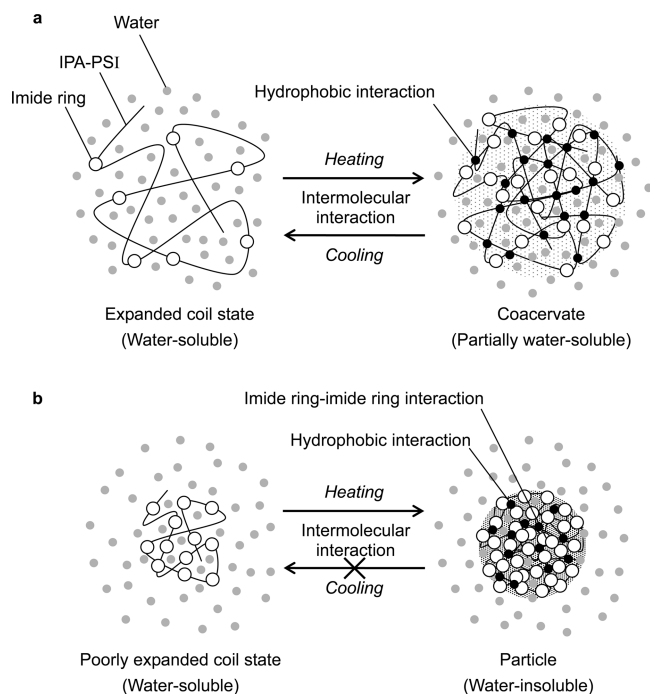


**FIGURE 3.** Colloid of IPA-PSI with various DS prepared by thermo-induced phase transition. (a) Optical microscopic image of 1 wt % aqueous solution of IPA-PSI with high DS, at 60 °C. (b) SEM image of IPA-PSI particulate prepared by irreversible phase transition of 1 wt % aqueous IPA-PSI solution. The particulate prepared by heating was washed by repeated centrifugation and subsequent dispersion in ultra pure water. The colloid was air-dried before observation.

The colloid induced by the phase transition of IPA-PSI was observed with an optical microscope equipped with a variable-temperature stage. Figure 3a is an image of an aqueous solution of IPA-PSI with high DS at 60 °C (above the cloud point). The figure clearly shows coacervate microdroplets, which formed thermoreversibly. By contrast, the colloid particles from IPA-PSI with low DS cannot be observed by optical microscopy, even though the solution became turbid at temperatures above the cloud point. This implies that very small colloid particles were created. The colloid particles formed by the thermoirreversible phase transition of IPA-PSI with low DS were recovered by centrifugation and resuspended in ultrapure water by agitation, confirming that the phase transition of IPA-PSI with low DS gave a solid product. Figure 3b shows a typical scanning electron microscopy image of the colloid particles prepared from IPA-PSI with low DS. The figure confirms that nanospheres several hundred nanometers in diameter were formed by the irreversible phase transition. Dynamic light scattering analysis of IPA-PSI solution after the irreversible phase transition also indicated the formation of nanospheres, in accordance with the SEM observation.

The strong dependence of cloud point on polymer concentration and the optical microscopic images provide useful information for speculation about the phase transition mechanism of IPA-PSI with high DS. Strong dependence of the cloud point on concentration has been reported for systems that show thermally induced phase separation, such as poly[(*N,N*-dimethylacrylamide)-*co*-(*N*-phenylacrylamide)], poly[(*N*-vinylamide)-*co*-(vinyl acetate)], and hydroxylated poly(*N*-isopropylacrylamide) (26–28). These polymers have hydrophilic segments that form hydrogen bonds with water molecules, and show no or only weak endothermic peaks at the cloud point in differential scanning calorimetry (DSC) scans. For the aqueous solution of IPA-PSI with a high DS, an endothermic peak was likewise not detected in the calorimetric study using DSC (see the Supporting Information). The origin of the endothermic peak at the cloud point is the disruption of hydrogen bonding between polymer and water molecules, and the conformational change of the polymer that results in the destruction and reconstruction of a hydrogen-bonded water network. Because of the hydrophilic polyamide backbone chain, the result obtained can be attributed to partial rather than complete dehydration.





**FIGURE 4.** Schematic illustration of phase transition of IPA-PSI. (a) phase transition mechanism of IPA-PSI with high DS showing reversible phase transition. (b) Phase transition mechanism of IPA-PSI with low DS showing irreversible phase transition.

These considerations lead to the proposed mechanism of the reversible phase transition of IPA-PSI with high DS shown in Figure 4a. Below the cloud point, IPA-PSI with high DS is in an expanded coil state because of its high hydrophilicity. Above the cloud point, the partially dehydrated IPA-PSI molecules associate each other through hydrophobic interactions. This means that IPA-PSI with high DS involves a lot of dehydrated water molecules through the hydrophilic backbone after the phase transition, forming coacervate droplets.

By contrast, the irreversible phase transition of IPA-PSI with low DS seems to occur by a different mechanism. Because conventional thermo-responsive polymers do not show the irreversible phase transition and do not generate stable nanospheres, IPA-PSI with low DS must possess interaction forces that are sufficiently strong to stabilize particle states even below the transition temperature. The nanospheres were washed with and stored in ultrapure water. Aqueous sodium hydroxide solution was then added dropwise to the colloidal solution with gentle agitation. The turbidity of the colloidal solution gradually disappeared, resulting in a transparent solution. It is known that sodium hydroxide cleaves the succinimide unit without breaking the bond to the IPA-PSI backbone, thus producing polyaspartate. The implication is that the imide rings somehow contribute to stabilizing the particle state of IPA-PSI, and it seems that there is an interaction between the distorted pentagonal rings. This is the basis of the proposed mechanism of the irreversible phase transition, which is shown in Figure 4b. IPA-PSI can dissolve in water at low temperature, but is in a poorly expanded coil state due to its low hydrophilicity. As temperature increases, the molecule is hydrophobized by

dehydration, and the hydrophobized molecules are aggregated intermolecularly by hydrophobic interaction. Above the cloud point, IPA-PSI molecules with low DS have few hydrogen-bonding interactions with water molecules and can form solid particles. The interaction between succinimide rings stabilizes the IPA-PSI conformation, resulting in stable particle even below the cloud point.

Generation of a novel biodegradable thermoresponsive polymer whose function can be altered by changing its composition, as described above, enables construction of a general purpose system. IPA-PSI can be chemically modified because the remaining succinimide units can react with nucleophiles. Therefore, IPA-PSI has the potential to replace conventional nondegradable thermo-responsive polymers in fields such as medicine, biology as well as environmental science if the phase transition temperature is tuned around 37 °C. Thus, IPA-PSI may be useful in applications such as drug delivery systems or separation technology. Furthermore IPA-PSI with low DS can be utilized in a novel process for preparing biodegradable nanospheres. This method has several advantages compared with conventional methods of preparation of biodegradable nanospheres. By contrast with most conventional methods, this simple method requires no toxic organic solvent (29, 30). The organic solvent-free process obviates the need for an organic solvent removal process, thus saving energy, reducing organic solvent waste, and eliminating hazards associated with residual organic solvent in drug, food, and environmental applications. The versatile biodegradable thermoresponsive polymer that we report here has the potential for medical, biological, and environmental applications.

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**Supporting Information Available:** Experimental procedure to synthesize IPA-PSI derivatives, transmittance curve, and cloud point determination, glass transition temperature, and a DSC result (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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