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Biodegradable thermoresponsive poly(amino acid)s

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Reaction of poly(succinimide) with a mixture of 5-aminopentanol and 6-aminohexanol produced new thermoresponsive polymers based on biodegradable poly(amino acid)s, poly(*N*-substituted α/β-asparagine)s, showing a clear LCST in water.

Recently, smart polymers, stimulus-responsive polymers, are becoming increasingly attractive for biotechnology and medicine.[1] Among them, thermoresponsive polymers have been widely investigated for various applications such as drug delivery, biomimetic actuators, chromatographic separations, gene-transfection agents, and immobilized biocatalysts.[2]

Poly(*N*-isopropylacrylamide) (PNIPAAm) is one of the most typical thermoresponsive polymers. PNIPAAm exhibits a rapid and reversible hydration–dehydration change in response to small temperature cycles around its lower critical solution temperature (LCST).[3] For development of intelligent materials based on PNIPAAm, introduction of functional groups was examined by copolymerization techniques. However, the sensitivity of temperature response was often reduced in aqueous media with increasing the composition of the functional comonomer.[4] Furthermore, PNIPAAm is toxic and non-biodegradable, resulting in limitation of its use in medical fields.

Some amphiphilic block copolymers are known to show phase transitions in water. A block copolymer consisting of biodegradable poly(lactic acid) and non-biodegradable poly(ethylene oxide) was developed as an injectable thermoresponsive polymer.[5] However, precise control over the molecular architecture was required for control of the thermoresponsive properties.

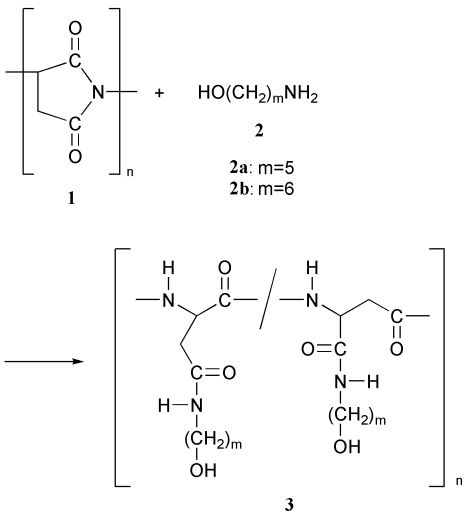
Amino acid-based polymers have been remarkably developed owing to their wide potential applications for biocompatible and biodegradable materials.[6] Some of these polymers have unique properties and functions derived from the amino acid moiety. Poly(aspartate), a poly(amino acid) with a carboxylate side chain, which is synthesized by thermal polymerization of aspartic acid followed by alkaline hydrolysis, has received much attention as a new, useful class of biodegradable, water-soluble polymeric materials.[7]

In the preparation of biodegradable poly(aspartate), poly(succinimide) (**1**) is obtained as an intermediate product, which is readily subjected to reaction with various nucleophiles. It was reported that alkaline hydrolysis of **1** produced poly(aspartate) with a mixed structure of α and β units.[7] This study deals with synthesis and properties of new biodegradable thermoresponsive poly(amino acid)s. Poly(succinimide) **1** was reacted with amino alcohols (**2**) to give poly(*N*-substituted α/β-asparagines) (**3**) (Scheme 1). An appropriate combination of amino alcohols produced **3** showing a sharp LCST.

Synthesis of **3** was carried out by using an excess of **2** for **1** at 50 °C in anhydrous *N*,*N*-dimethylformamide (DMF) for 24 h,† in which the quantitative introduction of the amino alcohol moiety was achieved. The polymer structure of **3** was confirmed by ^{1}H NMR. The reaction of **1** and **2a** produced a polymer with M_n of 1.9 × 10^4 and M_w/M_n of 2.8 in 85% yield, which was completely soluble in water in the range from 0 to 100 °C. On the other hand, a combination of **1** and **2b** produced a polymer insoluble in water at any temperature.

Thus, a mixture of **2a** and **2b** was employed as nucleophile. Four samples with different contents of the amino alcohols were synthesized: polymer A, content of **2a** = 50%; polymer B, content of **2a** = 60%; polymer C, content of **2a** = 70%; polymer D, content of **2a** = 80%. In all cases, the polymer was obtained in high yields and the content of **2a** in **3** determined by ^{1}H NMR was very close to the feed ratio.

Fig. 1 shows the temperature dependence of light transmittance of 1 wt% aqueous solution 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 90% transmittance of the polymer solution during the heating process. The LCST was in the range from 23 to 44 °C and



Scheme 1

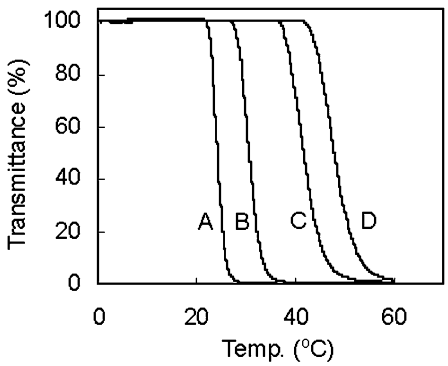


Fig. 1 Effect of temperature on light transmittance of 1 wt% aqueous solution of **3** (polymers A–D) on the heating process. The measurement was performed at 500 nm at a heating rate of 1 °C min^{-1}.

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linearly increased as a function of content of **2a** (correlation coefficient = 0.99) (Fig. 2). These data indicate that the LCST could be controlled by the composition ratio of **2a** and **2b** in **3**.

Fig. 3 shows the temperature dependence of the size of polymer A in water, determined by dynamic light scattering. Below 25 °C, the diameter was less than 10 nm, on the other hand, the diameter became larger than 200 nm above 30 °C. These data suggest that the phase transition of **3** took place in water near LCST, yielding nanoparticles with diameter of *ca.* 250 nm.

In conclusion, new thermoresponsive polymers based on biodegradable poly(amino acid)s were developed. Poly(*N*-substituted α/β-asparagine) **3** showing a sharp LCST was prepared by the reaction of **1** with a mixture of **2a** and **2b**. A linear relationship between content of **2a** and LCST was observed.

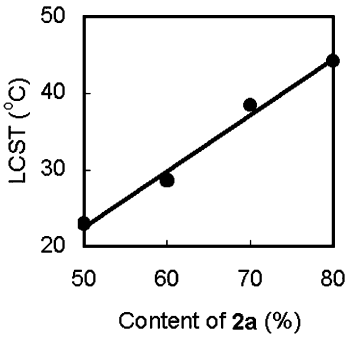

Fig. 2 Relationship between content of **2a** in **3** and LCST.

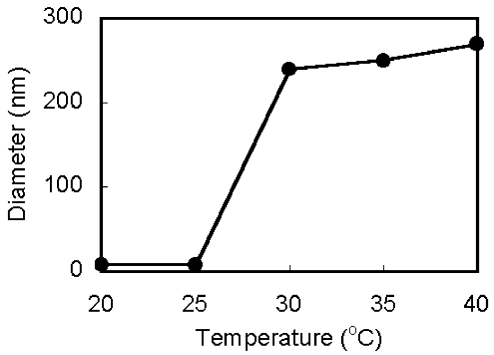

Fig. 3 Temperature dependence of the diameter of **3** (polymer A) in water.

The present thermoresponsive poly(amino acid)s have a hydroxy group in the side chain. Therefore, functional molecules such as drugs and probes can be easily introduced, which may be useful for various applications such as drug delivery systems (DDS) and bioconjugation. Further studies on new thermosensitive polymeric materials based on poly(*N*-substituted α/β-asparagine) are under way in our laboratory.

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Notes and references

† A typical run of polymer synthesis was as follows (polymer A). **1** (0.49 g, 5.0 mmol of succinimide unit), **2a** (0.52 g, 5.0 mmol), and **2b** (0.59 g, 5.0 mmol) were dissolved in 7.5 mL of anhydrous DMF under argon. The mixture was kept at 50 °C for 24 h. The reaction mixture was subjected to purification by dialysis (cut-off molecular weight = 1×10^3) against water several times. The remaining solution was lyophilized to give **3** (0.92 g, 89% yield). ^{1}H NMR (DMSO-d$_6$) δ 1.2–1.6 (br, $CH_2CH_2CH_2$), 2.9–3.1 (br, $NHCH_2CH_2$), 3.3–3.6 (br, $C(=O)CH_2CHC(=O)$), 4.2–4.8 (br, $C(=O)CH_2CHC(=O)$, CH_2OH), 7.5–8.5 (br, NH).

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