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Coil-Globule Transition and/or Coacervation of Temperature and pH Dual-Responsive Carboxylated Poly(*N*-isopropylacrylamide)

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We now report the stimuli-responsive behavior of an ionic poly(*N*-isopropylacrylamide)-based functional polymer, poly(NIPAAm-*co*-2-carboxyisopropylacrylamide) (poly(NIPAAm-*co*-CIPAAm)), which has carboxyl groups on the isopropyl side chains. The poly(NIPAAm-*co*-CIPAAm) exhibits a sensitive dehydration involving a coil-globule transition in acidic media above the lower critical solution temperature, while it induces an incomplete dehydration and shows coacervate formation when the carboxylic groups of the CIPAAm units are deprotonated. Therefore, by using the poly(NIPAAm-*co*-CIPAAm), we can first demonstrate that the dynamically tunable switching system between the coil-globule transition and the coacervation of thermoresponsive polymers can be successfully achieved by controlling the external stimuli, such as the pH change.

KEY WORDS: Stimuli-Responsive Polymers / Poly(*N*-isopropylacrylamide) / Lower Critical Solution Temperature / Coil-Globule Transition / Coacervation /

Thermoresponsive polymers have been intensively investigated and utilized in various fields including nanotechnology and biomedical applications because they can change their physical or chemical properties in response to a small temperature change.[1–3] In particular, poly(*N*-isopropylacrylamide) (PNIPAAm) is one of the most studied thermoresponsive polymers and is very attractive in terms of both fundamental and practical aspects.[4–7] In order to elucidate the phase transition mechanism of the thermoresponsive polymers, many studies have investigated the PNIPAAm aqueous system using several kinds of methods, such as spectroscopic or calorimetric analyses.[8–12] During the phase transition at the lower critical solution temperature (LCST), the PNIPAAm causes a sensitive dehydration behavior and exhibits a drastic conformational change, *i.e.*, a "coil-globule transition," leading to the formation of macroscopic aggregates due to a hydrophobic interaction among the collapsed polymer chains.[13,14] However, not all of the thermoresponsive polymers show a thermoresponsive behavior involving the coil-globule transition, as in the case of the PNIPAAm. It has been reported that some thermoresponsive polymers exhibit a coacervate formation above the LCST.[15–17] Such temperature-responsive polymers cannot cause the significant dehydration behavior.[15] Instead, they undergo incomplete dehydration and associate with each other possessing a large number of water molecules, resulting in the formation of micrometer-scale "coacervate droplets."

It is very important to demonstrate whether one thermoresponsive polymer exhibits the coil-globule transition or the coacervation. As already mentioned, the coil-globule transition-type thermoresponsive polymers can cause a drastic dehydration and their cross-linked hydrogel can also undergo a sensitive volume phase transition (swelling-deswelling) behavior. On the other hand, the coacervation-type polymers cannot undergo a drastic conformational change, or the sensitive volume phase transition in the form of hydrogels, since their dehydration behaviors are incomplete. However, because of their insufficient dehydration behaviors, the coacervation-type polymers do little or no damage to biological macromolecules or physiologically active substances, and the coacervate droplets induced in such polymers can enrich and include proteins and nucleic acids without losing their functions, due to the considerably high water content in the coacervate phase.[18,19] In contrast, the coil-globule transition-type polymers can possibly do serious damage to some bioactive molecules due to their significant dehydration behaviors and strong hydrophobicity. Therefore, it is important to determine which type of thermoresponsive polymers should be used, depending on the intended use.

In our previous research, we newly designed PNIPAAm-based stimuli-responsive polymers, such as an ionic poly(NIPAAm-*co*-2-carboxyisopropylacrylamide) (poly(NIPAAm-*co*-CIPAAm)) and a nonionic poly(NIPAAm-*co*-2-hydroxyisopropylacrylamide) (poly(NIPAAm-*co*-HIPAAm)), in order to develop the PNIPAAm by introducing various functional groups onto the isopropyl side chains.[20–22] Moreover, during the course of the studies on the nonionic poly(NIPAAm-*co*-HIPAAm), it was suggested that the poly(NIPAAm-*co*-HIPAAm)s with a low hydrophilic HIPAAm comonomer content show the coil-globule transition, while the copolymers with a high content exhibit the coacervate formation.[21,23,24] In other words, we demonstrated that the coil-globule transition or the coacervation of the PNIPAAm-based thermoresponsive polymers can be modulated by controlling the HIPAAm comonomer content. Furthermore, these results also indicated the possibility that introducing a certain hydrophilic factor level into a thermoresponsive polymer leads to the thermor-

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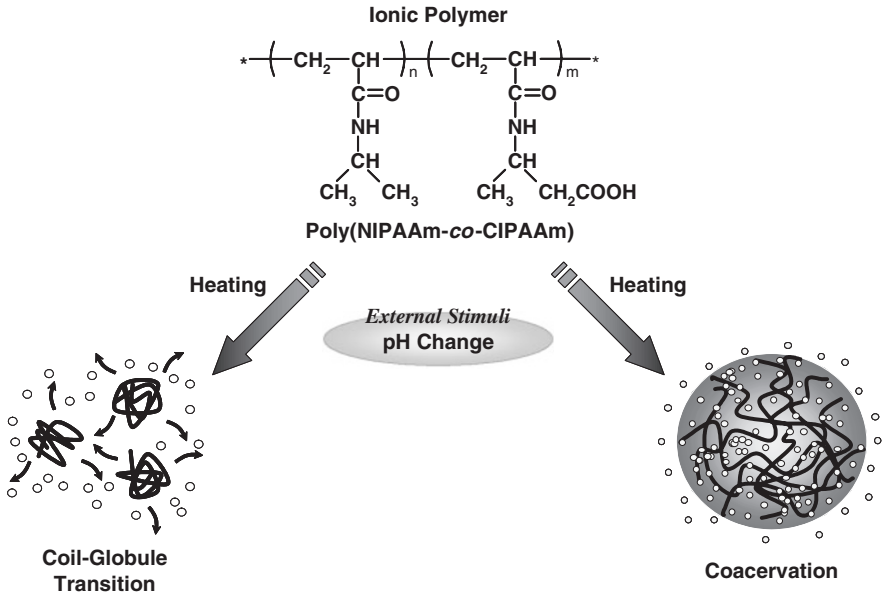

Figure 1. Coil-globule transition and/or coacervation of poly(NIPAAm-*co*-CIPAAm) in response to temperature and pH changes.

esponsive-type coacervation. As for the poly(NIPAAm-*co*-HIPAAm), the HIPAAm comonomer content serves as a controlling factor to determine whether the coil-globule transition or the coacervation occurs. However, in this case, the structure of the polymer itself needs to be changed in order to switch between the coil-globule transition and the coacervation because of involving the comonomer composition. For more easily achieving the switching system between the coil-globule transition and the coacervation, a dynamically controllable switching system should be desired.

In this study, we therefore investigated whether one can determine the coil-globule transition or the coacervation of thermoresponsive polymers not by changing the structures of the polymers themselves, but by external stimuli using the ionic poly(NIPAAm-*co*-CIPAAm) (Figure 1). Because the poly(NIPAAm-*co*-CIPAAm) has ionic carboxyl groups on the side chains, its hydration state could be modulated by a pH change of the aqueous medium. Moreover, randomly and homogeneously arranged CIPAAm units in each poly(NIPAAm-*co*-CIPAAm) chain could also help to change the hydration state of the overall polymer chains in response to the pH change.[25] Accordingly, the dynamically tunable switching system between the coil-globule transition and the coacervation is expected to be demonstrated using the poly(NIPAAm-*co*-CIPAAm) only by external stimulus, *i.e.*, the pH change.

EXPERIMENTAL

Materials

N-Isopropylacrylamide (NIPAAm) was kindly provided by Kohjin (Tokyo, Japan) and recrystallized from hexane. 2-Carboxyisopropylacrylamide (CIPAAm) monomer was synthesized as in our previous study.[20] 2,2′-Azobis(isobutyronitrile) (AIBN) was obtained from Wako Pure Chemical Industries (Tokyo, Japan) and recrystallized from ethanol. All other reagents were used as received.

Copolymer Synthesis

The poly(NIPAAm-*co*-CIPAAm)s were prepared in dry *N*,*N*-dimethylformamide (DMF) by free radical copolymerization with AIBN as the initiator. Various compositions of the monomers, NIPAAm and CIPAAm, and AIBN (0.1 mmol: 1.0 mol % relative to the total monomers) were dissolved in DMF and the solutions were degassed by a freezing and thawing process. The polymerization was performed at 60 °C for 24 h, and the resultant polymer solutions were dialyzed against ethanol and then distilled water for 1 week. The dialyzed solutions were lyophilized and the copolymers were obtained as a white powder. The comonomer compositions of the resultant copolymers were estimated by ^{1}H NMR (JEOL JNM-GSX400, 400 MHz spectrometer) and are summarized in Table I. In this paper, the poly(NIPAAm-*co*-CIPAAm)s are abbreviated $N_{100-x}C_x$, where $N_{100-x}C_x$ denotes the poly(NIPAAm-*co*-CIPAAm) containing X mol % of CIPAAm.

LCST Measurements

The LCSTs of the copolymer solutions (1.0 mg/mL) were determined using a UV-vis spectrophotometer (Jasco V-550

Table I. Preparation of poly(NIPAAm-*co*-CIPAAm) with various CIPAAm compositions

Code	In Copolymer (mol %)[a]		Yield (%)
	NIPAAm	CIPAAm	
$N_{97}C_3$	97	3	97
$N_{96}C_4$	96	4	94
$N_{95}C_5$	95	5	96

[a]Determined by ^{1}H NMR.

spectrophotometer, Tokyo, Japan) equipped with a temperature controller. The copolymers were dissolved in various pH media, which were adjusted using a 0.01 M HCl solution, 0.01 M NaOH solution, or phosphate buffer solution (PBS, pH 7.4). In addition, the salt concentrations of these solutions were adjusted to 150 mM with sodium chloride (NaCl). The transmittances of the copolymer solutions were recorded as a function of temperature at a 500 nm wavelength and at the heating rate of 1.0 °C/min. The LCSTs were defined as the temperature at 50% light transmittance.

Microscopic Observation

Optical images of the copolymer solutions (1.0 mg/mL) were recorded above the LCSTs using a Nikon ECLIPSE TS 100 microscope equipped with a Digital Sight DS-5M, Nikon (Tokyo, Japan).

Dynamic Light Scattering Measurements

The size of the coacervate droplets induced in the copolymer solutions (1.0 mg/mL) was evaluated by dynamic light scattering (DLS). The DLS measurements were carried out at 5 °C higher than the LCSTs of each copolymer solution using a DLS instrument (FPAR-1000HL, Otsuka Electronics Co., Ltd., Osaka, Japan) with a vertically polarized incident beam of 658 nm wavelength from a semiconductor laser. A scattering angle of 90° was used in these measurements.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measurements were carried out using a DSC 6100 calorimeter (Seiko Instruments, Tokyo, Japan). The copolymers were dissolved in a 0.1 M HCl or NaOH solution containing salt (150 mM NaCl) at the concentration of 50 mg/mL. The polymer solutions were placed in silver pans and the pans were then completely sealed to prevent the water from evaporating. The measurements were taken at the heating rate of 2.0 °C/min from 10 to 90 °C, and the reproducibility was checked by running three experiments.

RESULTS AND DISCUSSION

Transmittance Measurement of Poly(NIPAAm-*co*-CIPAAm) in Aqueous Media of Various pHs

The turbidity of the poly(NIPAAm-*co*-CIPAAm) was monitored as a function of temperature in various pH media. Figure 2 shows the transmittance curves of the $N_{97}C_3$ copolymer in aqueous HCl and NaOH solutions in the absence and presence of salt. In the HCl solution (pH 2.1), the $N_{97}C_3$ copolymer exhibits a very sensitive transmittance change regardless of the absence or presence of salt, while it did not show a thermoresponse in the NaOH solution (pH 11.9) without adding salt. Because the carboxylic groups of the poly(NIPAAm-*co*-CIPAAm) are dissociated and ionized in the basic solution, a significant increase in hydrophilicity of the polymeric chains and the electrostatic repulsion among the ionized carboxylic groups make it difficult to induce the dehydration and the following aggregation among the polymer chains.[26] However, by adding 150 mM NaCl to the solution, the $N_{97}C_3$ copolymer could exhibit a clear transmittance change due to the salting-out effect and/or suppression of the electrostatic repulsion caused by the counter ion.[27] Therefore, the salt concentrations of the aqueous media were adjusted to 150 mM in the subsequent experiments.

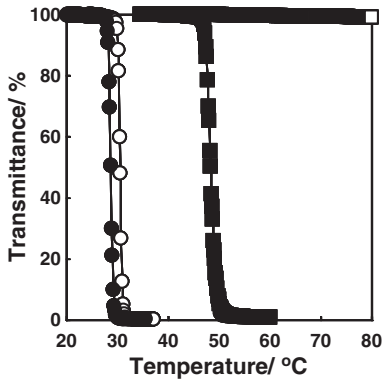

Figure 2. Transmittance curves of $N_{97}C_3$ (1.0 mg/mL) in 0.01 M HCl (circles) and NaOH (squares) solutions in the absence (open symbols) and presence (closed symbols) of 150 mM NaCl.

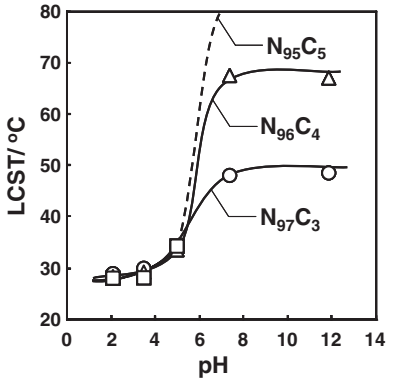

Figure 3. LCSTs for poly(NIPAAm-*co*-CIPAAm) aqueous solutions (1.0 mg/mL) as a function of pH. The salt concentrations of these solutions were adjusted to 150 mM with sodium chloride (NaCl).

Figure 3 indicates the LCSTs of the copolymers as a function of pH. The LCST gradually increased with the increasing pH and drastically shifted to a higher temperature around pH 6, which is almost consistent with the value of the apparent acidic dissociation constant (pK_a) of the poly(NIPAAm-*co*-CIPAAm).[28] That is, the dissociation of the carboxylic groups on the isopropyl side chains are drastically promoted around pH 6, which induces the ionic hydration of the polymer chains. The abrupt enhancement in the hydrophilicity of the polymeric chains by the pH change results in a discontinuous increase in the LCST, reflected as the clear pH-responsive behavior of the poly(NIPAAm-*co*-CIPAAm). Moreover, variation in the LCST for the $N_{96}C_4$ copolymer caused by the pH change is much greater than that for the $N_{97}C_3$ copolymer, and the $N_{95}C_5$ copolymer did not show any LCST behavior below 100 °C at atmospheric pressure when the pH is

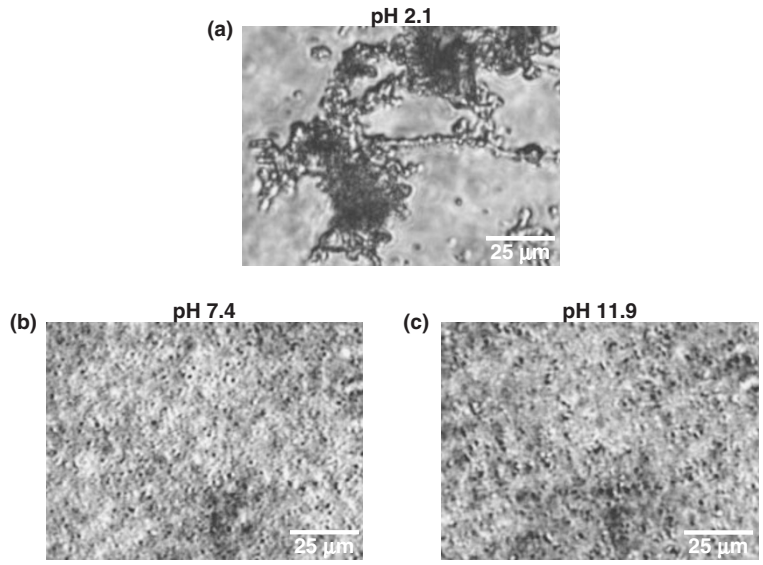

Figure 4. Micrographs of poly(NIPAAm-*co*-CIPAAm) (1.0 mg/mL) in various pH media above the LCSTs (at 70 °C).

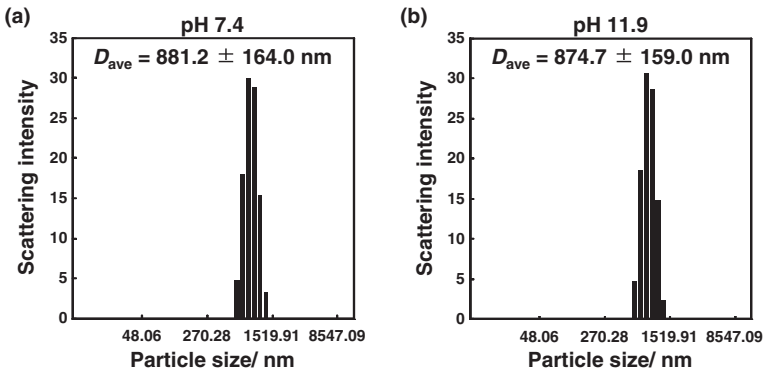

Figure 5. DLS histograms of coacervate droplets induced in $N_{97}C_3$ aqueous solutions (1.0 mg/mL) at (a) pH 7.4 and (b) pH 11.9 above the LCSTs.

above the pK_a of the copolymer. These results indicated that the slight difference in the CIPAAm content exerts a significant influence on the thermoresponsive behavior of the copolymer under the condition that the carboxylic groups are deprotonated.

Coil-Globule Transition and/or Coacervation of Poly(NIPAAm-*co*-CIPAAm) in Response to pH

Figure 4 shows micrographs of the $N_{97}C_3$ copolymer in aqueous media with various pHs. By heating the polymer solution above the LCST, macroscopic aggregates were observed in the acidic solution (Figure 4a), which is identically induced in the PNIPAAm aqueous system. This result suggests that the poly(NIPAAm-*co*-CIPAAm) causes a coil-globule transition under acidic conditions, and then the dehydrated/collapsed polymer chains aggregate with each other due to the hydrophobic interaction. Very interestingly, however, the copolymer showed a coacervate formation instead of the macroscopic aggregate above the LCST in the basic solution (Figure 4c). It should also be noted that the coacervate droplets could be observed at pH 7.4 (Figure 4b), which is slightly higher than the pK_a value of the copolymer. Additionally, similar results were observed in the $N_{96}C_4$ copolymer aqueous system. Therefore, these results strongly indicate that the dissociation state of the carboxylic groups on the side chains has a significant effect on the thermoresponsive behavior of the poly(NIPAAm-*co*-CIPAAm): coil-globule transition or coacervation.

The particle sizes of the thermally induced coacervate droplets from the poly(NIPAAm-*co*-CIPAAm) aqueous solution were determined by DLS measurements. Figure 5 shows the DLS histograms of the coacervate droplets induced in the $N_{97}C_3$ copolymer solution at pHs 7.4 and 11.9 above the LCSTs. At both pHs, their average diameters were about 900 nm and single peaks were observed. Figure 4b and 4c also represent the comparatively monodispersed coacervate droplets although the measurement temperatures were different from those of the DLS because of a problem in the measurement. (The DLS measurements of the copolymer solutions were carried out at 5 °C higher than the LCSTs of each copolymer solution. On the other hand, the micrographs were taken at 70 °C because it was difficult to clearly take a picture of the

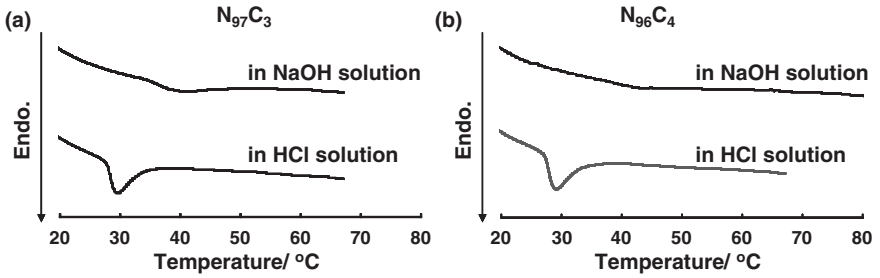

Figure 6. DSC thermograms of (a) $N_{97}C_3$ and (b) $N_{96}C_4$ (50 mg/mL) in 0.1 M HCl or NaOH solution containing 150 mM NaCl.

coacervate droplets at 5 °C higher than the LCSTs.) Therefore, we demonstrated that the poly(NIPAAm-*co*-CIPAAm) can form fine coacervate droplets above the LCSTs under the condition that the carboxylic groups on the side chains are in the deprotonated state. In general, most of the thermally induced coacervate droplets reported up to now have broad size distributions.[16,29] The fine coacervate droplets induced in the thermoresponsive poly(NIPAAm-*co*-CIPAAm) should be due to a random comonomer sequence and a homogeneous comonomer content in each copolymer chain, which is suggested by the estimated values of the monomer reactivity ratios.[21,25]

It has been reported that the significant difference between the coil-globule transition and the coacervation of the thermoresponsive polymers is the dehydration behavior induced during the phase transition and/or separation.[15,29] Therefore, we carried out the DSC measurements of the poly(NIPAAm-*co*-CIPAAm) aqueous solutions. Figure 6 shows the DSC thermograms of the copolymers in acidic and basic solutions. In the acidic solution, both the $N_{97}C_3$ and $N_{96}C_4$ copolymers display the well-defined endothermic peak arising from the sensitive dehydration, which could be observed at the temperature corresponding to the LCST determined by the transmittance measurements (Figure 3). The enthalpies of transition, ΔH_t, estimated by the peak area of the DSC thermograms, were 5.28 and 5.48 kJ/mol of the monomer units for $N_{97}C_3$ and $N_{96}C_4$, respectively. These ΔH_t values were almost the same as that for the PNIPAAm,[24,30] indicating that the poly(NIPAAm-*co*-CIPAAm) can exhibit a sensitive dehydration similar to the PNIPAAm under the condition that the carboxylic groups are protonated. Due to their sensitive dehydration, a gain in entropy, resulting from the release of structured waters existing around the hydrophobic moieties of the polymers, can satisfactorily compensate for a loss of entropy arising from the collapse of the polymer chains.[31] Hence, the polymer chains can undergo the coil-globule transition. However, in alkali solution, no clear endothermic peaks could be observed for both the $N_{97}C_3$ and $N_{96}C_4$ copolymers. The deprotonated carboxylic groups make the polymeric chains significantly hydrophilic, thus difficult to induce a sensitive dehydration. Their incomplete dehydration behavior leads to a decrease in the gain of entropy, making it impossible to compensate for the loss of entropy due to the collapse of the polymer chains. Consequently, the polymer chains cannot cause the coil-globule transition and the following aggregation among the hydrophobized species themselves. Instead, since the polymer chains induce the incomplete dehydration in no small way, partially dehydrated polymer chains associate with each other through a hydrogen bond and a hydrophobic interaction, leading to the formation of the coacervate droplets containing a large number of water molecules.[24] Consequently, it was suggested that the poly(NIPAAm-*co*-CIPAAm) can show both the coil-globule transition and the coacervation in response to temperature and pH changes (Figure 7). In other words, by using the ionic thermoresponsive poly(NIPAAm-*co*-CIPAAm), we can demonstrate the dynamically tunable switching system between the coil-globule transition and the coacervation of the thermoresponsive polymers by external stimuli, *i.e.*, the pH change. Based on Figure 3, it has been suggested that the deprotonated CIPAAm units in the copolymer chains have a strong influence on the thermoresponsive behavior of the copolymer. That is, only 3 or 4 mol % of the CIPAAm unit can dramatically vary the hydration states of the copolymer chains in response to the pH change, making it possible to induce a dynamically tunable switching system between the coil-globule transition and the coacervation. Furthermore, it should also be noted that the random comonomer sequence and the homogeneous comonomer content in each poly(NIPAAm-*co*-CIPAAm) chain can significantly contribute to realizing the dynamic switching system as well as to the formation of the fine coacervate droplets. Randomly and homogeneously arranged CIPAAm units in each copolymer chain enable one to modulate the hydration state of the overall polymeric chains in response to a pH change. It has been reported by other research group that the copolymer having a nonrandom comonomer sequence could not exhibit a clear phase separation behavior and even formed the micelle-like structure in some cases.[29] Additionally, we have confirmed that the dynamic switching system could not be achieved using the nonionic poly(NIPAAm-*co*-HIPAAm) aqueous system by the pH change since the thermoresponsive behavior of the poly(NIPAAm-*co*-HIPAAm) does not depend on the pH of the aqueous medium.[21,24]

Direct Globule-*to*-Coacervate and Coacervate-*to*-Globule Changes in Poly(NIPAAm-*co*-CIPAAm)

As shown in Figure 7, we demonstrated that the dynamic switching system between the coil-globule transition and the

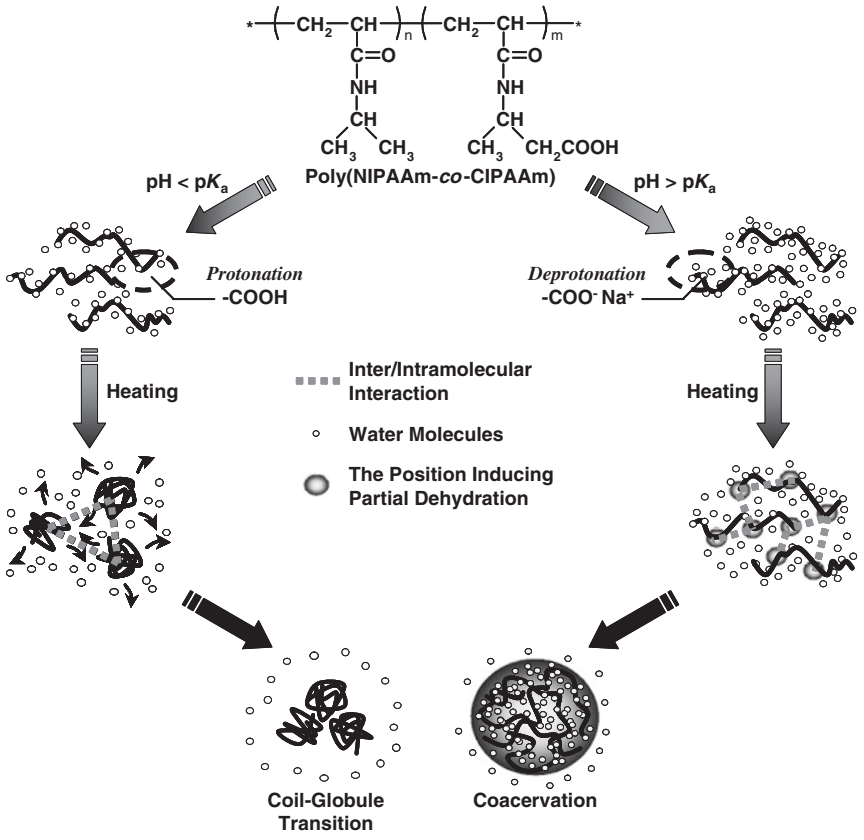

Figure 7. Schematic illustration of stimuli-responsive mechanism of poly(NIPAAm-*co*-CIPAAm) in response to temperature and pH changes.

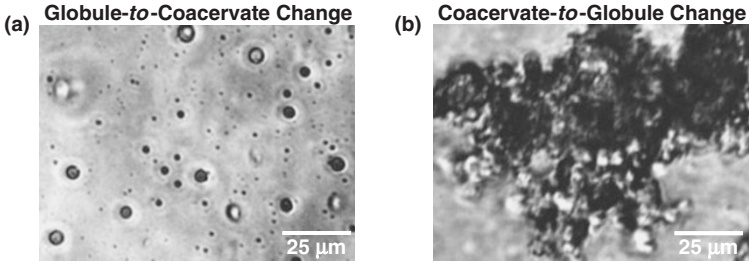

Figure 8. Micrographs of (a) globule-*to*-coacervate and (b) coacervate-*to*-globule changes in $N_{97}C_3$ aqueous solutions (1.0 mg/mL).

coacervation of the thermoresponsive polymers can be accomplished by controlling the hydration state due to the pH change followed by heating the solution above the LCST. In this case, the polymeric chains in a hydrated random coil state show either the coil-globule transition or the coacervate formation. Next, we therefore investigated whether one can directly induce the globule-*to*-coacervate or coacervate-*to*-globule change in the thermoresponsive polymers by external stimuli. In order to examine the globule-*to*-coacervate change in the poly(NIPAAm-*co*-CIPAAm), the following experiments were carried out: the $N_{97}C_3$ copolymer dissolved in a 0.01 M HCl solution containing 150 mM NaCl was heated above the LCST (70 °C) and incubated for 2 min to cause the coil-globule transition. This state now corresponds to Figure 4a. In the globule state, a small amount of a 1.0 M NaOH solution heated to 70 °C was added to the copolymer solution so as to change the solution from acidic to basic, keeping the temperature at 70 °C. After incubation for 2 min, a microscopic observation was then performed. The experiments for the coacervate-*to*-globule change were performed in a similar way except for replacing the HCl solution with NaOH solution and vice versa. In these experiments, it should be noted that before and after adding the 1.0 M NaOH or HCl solution, drastic pH changes were steadily induced and the polymer concentration (1.0 mg/mL) and the salt concentration of the aqueous medium were almost kept constant. Figure 8a and 8b show the results of the experiments for the globule-*to*-coacervate and coacervate-*to*-globule changes, respectively. In Figure 8b, macroscopic aggregates were observed, suggesting that a coacervate-*to*-globule change could be caused by the pH change. An abrupt

pH change from basic to acidic induced the protonation of the carboxylic groups of the CIPAAm units, which might promote the additional dehydration and lead to aggregates among the hydrophobized collapsed chains. Moreover, in Figure 8a, coacervate droplets could be clearly observed, suggesting that a globule-*to*-coacervate change could also be induced. For us, this result was unexpected because we had speculated that it is unlikely for the dehydrated and collapsed polymer chains to re-hydrate and associate with each other to form the coacervate droplets. However, the spherical droplets were obviously formed although their appearance were quite different from that of the coacervate droplets induced in the hydrated copolymer by heating the solution above the LCST (Figure 4c). In our previous research, we demonstrated that the dehydration process of thermoresponsive polymers during the phase separation is a very important to determine the character of the thermoresponsive-type coacervate droplets, such as their sizes and distribution.[24] Therefore, it is considered to be very difficult that the once dehydrated polymer chains in the globule state can form the fine coacervate droplets after just re-hydration caused by the pH change. That is why the appearance of the coacervate droplets produced by the globule-*to*-coacervate change could remarkably differ from that induced in the hydrated copolymer by heating. However, it was demonstrated that the direct globule-*to*-coacervate and coacervate-*to*-globule changes in the thermoresponsive polymers could be dynamically accomplished by external stimuli, such as the pH change in the aqueous media.

CONCLUSIONS

In this study, we could first demonstrate a dynamically tunable switching system between the coil-globule transition and the coacervation of a thermoresponsive polymer by external stimulus, *i.e.*, a pH change, using the ionic thermoresponsive poly(NIPAAm-*co*-CIPAAm). The thermoresponsive behaviors of the copolymers were sensitive to a pH change in the aqueous medium. That is, their LCSTs drastically varied around the pH, almost corresponding to the value of the apparent acidic dissociation constant (pK_a) of the copolymers. These results suggest that their thermoresponsive behaviors closely depend on the dissociation state of the carboxylic groups on the isopropyl side chains of the CIPAAm units. Under acidic conditions, the copolymers caused the sensitive dehydration and then formed macroscopic aggregates due to the hydrophobic interaction among the dehydrated polymer chains, indicating that the copolymers exhibit the coil-globule transition as in the case of the aqueous PNIPAAm system. However, when the pH of the aqueous medium is over the pK_a value of the copolymers, their dehydration behaviors become incomplete and the fine coacervate droplets could be observed above the LCSTs. In other words, the copolymers show a thermoresponsive-type coacervate under the condition that the carboxylic groups are in a dissociated state. These results strongly demonstrated that the pH of the aqueous medium determines the dissociation state of the carboxylic groups of the randomly arranged CIPAAm units on the copolymer chains, leading to control of the hydration states of the overall polymeric chains. Consequently, the poly(NIPAAm-*co*-CIPAAm) could show both the coil-globule transition and the coacervation in response to the pH change. It should also be noted that the random comonomer sequence and the homogeneous comonomer content in each poly(NIPAAm-*co*-CIPAAm) chain can significantly contribute to realizing a dynamically tunable switching system as well as the formation of the fine coacervate droplets. Moreover, by changing the pH of the aqueous medium and keeping the temperature above the LCST, it was demonstrated that the direct globule-*to*-coacervate and coacervate-*to*-globule changes in a thermoresponsive polymer could also be dynamically achieved. There are some significant differences in the characters of the thermoresponsive polymers between the coil-globule transition and the coacervation, such as the sensitivity of the thermoresponse, hydration state, and the effect on bioactive molecules including proteins and peptides. Thus, we consider that it is very important to demonstrate whether one thermoresponsive polymer shows the coil-globule transition or the coacervation, and then to determine which type of thermoresponsive polymers should be used depending on the intended use. Therefore, the dynamically and easily controllable switching system between the coil-globule transition and the coacervation of thermoresponsive polymers can be very significant.

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REFERENCES

1. P. S. Stayton, T. Shimoboji, C. Long, A. Chilkoti, G. Chen, J. M. Harris, and A. S. Hoffman, *Nature*, **378**, 472 (1995).
2. L.-Y. Chu, Y. Li, J.-H. Zhu, and W.-M. Chen, *Angew. Chem., Int. Ed.*, **44**, 2124 (2005).
3. D. L. Huber, R. P. Manginell, M. A. Samara, B.-I. Kim, and B. C. Bunker, *Science*, **301**, 352 (2003).
4. H. G. Schild, *Prog. Polym. Sci.*, **17**, 163 (1992).
5. S. Fujishige, K. Kubota, and I. Ando, *J. Phys. Chem.*, **93**, 3311 (1989).
6. J. Kim, M. J. Serpe, and L. A. Lyon, *J. Am. Chem. Soc.*, **126**, 9512 (2004).
7. L. Ionov, M. Stamm, and S. Diez, *Nano Lett.*, **6**, 1982 (2006).
8. Y. Maeda, T. Higuchi, and I. Ikeda, *Langmuir*, **16**, 7503 (2000).
9. Y. Ono and T. Shikata, *J. Am. Chem. Soc.*, **128**, 10030 (2006).
10. F. M. Winnik, *Macromolecules*, **23**, 233 (1990).
11. H. G. Schild and D. A. Tirrell, *J. Phys. Chem.*, **94**, 4352 (1990).
12. A. K. Lele, M. M. Hirve, M. V. Badiger, and R. A. Mashelkar, *Macromolecules*, **30**, 157 (1997).
13. M. Heskins and J. E. Guillet, *J. Macromol. Sci., Chem.*, **2**, 1441 (1968).
14. J. S. Scarpa, D. D. Mueller, and I. M. Klotz, *J. Am. Chem. Soc.*, **89**,

6024 (1967).
15. H. Miyazaki and K. Kataoka, *Polymer*, **37**, 681 (1996).
16. X. Yin and H. D. H. Stöver, *Macromolecules*, **36**, 9817 (2003).
17. T. Shimokuri, T. Kaneko, T. Serizawa, and M. Akashi, *Macromol. Biosci.*, **4**, 407 (2004).
18. N. L. Abbott, D. Blankschtein, and T. A. Hatton, *Macromolecules*, **24**, 4334 (1991).
19. H.-O. Johansson, G. Karlström, and F. Tjerneld, *Macromolecules*, **26**, 4478 (1993).
20. T. Aoyagi, M. Ebara, K. Sakai, Y. Sakurai, and T. Okano, *J. Biomater. Sci., Polym. Ed.*, **11**, 101 (2000).
21. T. Maeda, T. Kanda, Y. Yonekura, K. Yamamoto, and T. Aoyagi, *Biomacromolecules*, **7**, 545 (2006).
22. T. Yoshida, T. Aoyagi, E. Kokufuta, and T. Okano, *J. Polym. Sci., Part A: Polym. Chem.*, **41**, 779 (2003).
23. T. Maeda, K. Yamamoto, and T. Aoyagi, *J. Colloid Interface Sci.*, **302**, 467 (2006).
24. T. Maeda, M. Takenouchi, K. Yamamoto, and T. Aoyagi, *Biomacromolecules*, **7**, 2230 (2006).
25. T. Kanda, K. Yamamoto, and T. Aoyagi, *J. Photopolym. Sci. Technol.*, **18**, 515 (2005).
26. G. Chen and A. S. Hoffman, *Nature*, **373**, 49 (1995).
27. S. Beltran, J. P. Baker, H. H. Hooper, H. W. Blanch, and J. M. Prausnitz, *Macromolecules*, **24**, 549 (1991).
28. M. Ebara, M. Yamato, S. Nagai, T. Aoyagi, K. Kikuchi, K. Sakai, and T. Okano, *Surf. Sci.*, **570**, 134 (2004).
29. S. Sugihara, S. Kanaoka, and S. Aoshima, *Macromolecules*, **37**, 1711 (2004).
30. K. Otake, H. Inomata, M. Konno, and S. Saito, *Macromolecules*, **23**, 283 (1990).
31. H. Feil, Y. H. Bae, J. Feijen, and S. W. Kim, *Macromolecules*, **26**, 2496 (1993).

