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Effect of Urea on Phase Transition of Poly(*N*-isopropylacrylamide) Investigated by Differential Scanning Calorimetry

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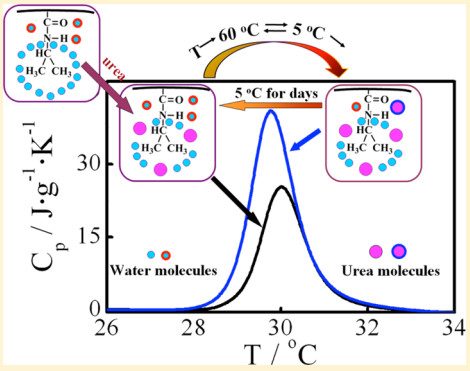
ABSTRACT: The effect of urea on the phase transition of PNIPAM was studied using differential scanning calorimetry (DSC). For a certain urea concentration, the enthalpy change of phase transition of poly(*N*-isopropylacrylamide) (PNIPAM) aqueous solution increases with the number of DSC cycles, presumably due to the displacement of water molecules bound to the amide groups of PNIPAM by urea molecules at the temperature higher than the lower critical solution temperature (LCST) of PNIPAM and causes the decrease in the absolute value of the exothermic heat related to the dehydration of hydrophilic groups and interactions of hydrophilic residues to around 0. Moreover, the enthalpy change decreases with the urea concentration during the heating process of the first DSC cycle, indicating the replacement of water molecules around the apolar isopropyl groups by urea molecules at the temperature lower than LCST, and the endothermic heat caused by the dehydration of apolar groups decreases. Furthermore, the urea molecules which replace the water molecules at high temperature can be replaced again by water molecules at the temperature lower than LCST, but this process needs several days to complete.



INTRODUCTION

Poly(*N*-isopropylacrylamide) (PNIPAM) is a thermally sensitive polymer which is soluble and adopts a coil conformation for linear polymer in aqueous solutions at low temperature and becomes insoluble at the temperature higher than its lower critical solution temperature (LCST ~ 32 °C), and its conformation changes to a globule one.[1] The phase transition of PNIPAM has received great attention not only due to the importance in polymer physics but also because of the potential applications for drug release[2−5] and separation sciences.[6,7] Until now, different techniques such as laser light scattering,[8−14] fluorescence spectroscopy,[15−17] tubidimetry,[18−22] differential scanning calorimetry (DSC),[19,21−29] infrared spectroscopy,[30−33] nuclear magnetic resonance,[34−36] and Raman spectroscopy[37−39] have been used to understand the phase transition of PNIPAM. For example, a single endothermic peak was observed during the phase transition of PNIPAM in aqueous solutions using DSC, indicating that the transition is mainly entropy-driven presumably due to the dehydration of ordered water molecules around apolar isopropyl groups and/or polar amide groups.[19,21−27,29,40−44] Grinberg et al. stated that the phase transition of PNIPAM hydrogels consists of the heat related to the dehydration of apolar groups, the heat related to the dehydration of polar groups, and the heat caused by the interactions (van der Waals and/or hydrogen bonding interactions).[27] Cho et al. used temperature modulated differential scanning calorimetry (TMDSC) to study the phase transition of PNIPAM aqueous solutions and suggested that the phase transition consists of at least two different thermal processes by resolving the single endothermic heat into an endothermic and exothermic peak.[28] However, Cho et al. also mentioned that the exact value of the enthalpy change for each process is difficult to obtain because the frequency region covered by TMDSC is limited (10^{-1}−10^{-2} Hz). Note that using Fourier transform infrared (FTIR) spectroscopy Maeda et al.[30] and Sun et al.[33] found that the band of asymmetric C—H stretching of the isopropyl groups shifts to lower wavenumbers at the higher temperature, indicating the dehydration of the isopropyl groups. Moreover, ~13% of the C═O groups from the amide groups of PNIPAM form intra- and/or interchain hydrogen bonds with the N—H groups, and the remaining C═O groups form hydrogen bonds with water molecules in the globule state, as stated by Maeda et al.[30] However, the enthalpy changes of the dehydration of isopropyl groups and amide groups are not fully understood because it is difficult to separate them from each other. Thus, a deep insight into the enthalpy change of PNIPAM aqueous solutions is still needed and will be beneficial to understanding the mechanism of phase transition.

On the other hand, urea as a widely used protein denaturant has received much attention.[45−50] The molecular mechanism of urea-induced denaturation is still an open question despite

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extensive studies. Two distinct mechanisms have been proposed. One is an indirect mechanism which suggested that urea molecules are presumed to disrupt the network structure of water molecules and lead to easy solvation of hydrophobic groups.[47,50] The other is a direct interaction mechanism; in this model, urea molecules directly interact with the proteins including the main chain and side groups by van der Waals interaction, hydrogen bonding interaction, and/or electrostatic interaction.[51−54] For example, using all-atom microseconds molecular dynamic simulations of hen lysozyme, Hua et al. stated that the stronger direct dispersion interaction between urea and protein backbone and side chains as compared to that for water causes the denaturation and supported the "direct mechanism".[54] To answer the question whether urea molecules interact directly with proteins, Sagle et al. used FTIR and Stokes radius measurements to study the interaction of urea with PNIPAM which serves as a simple model system, and they found that the decrease in the LCST of PNIPAM was related to the direct hydrogen bonding of urea to the amide groups of PNIPAM.[55]

To investigate the effect of urea on phase transition of PNIPAM and study the interaction between urea and PNIPAM, an ultrasensitive DSC was used in this study. Our study reveals that for a given certain urea concentration the enthalpy change of phase transition of PNIPAM aqueous solutions increases with the number of DSC cycles, indicating that water molecules bound to the amide groups of PNIPAM are displaced by urea molecules at the temperature higher than LCST. Moreover, during the heating process of the first DSC cycle, the enthalpy change decreases with the urea concentration, presumably due to the replacement of water molecules around the isopropyl groups at the temperature lower than the LCST. Therefore, with the addition of urea, the endothermic heat related to dehydration of the apolar isopropyl groups and the exothermic heat caused by the dehydration of the polar amide groups and interactions of hydrophilic residues might be observed, respectively.

EXPERIMENTAL SECTION

Materials and Sample Preparation. *N*-Isopropylacrylamide (Aldrich, 97%) was recrystallized three times from a benzene/*n*-hexane mixture prior to use. Poly(*N*-isopropylacrylamide) (PNIPAM) was synthesized by free radical polymerization in benzene with azobis(isobutyronitrile) (AIBN) as the initiator. The PNIPAM sample was further fractionated by a dissolution/precipitation process in a mixture of dry acetone and dry hexane at room temperature. The detail of synthesis can be found elsewhere.[10,11,44] The weight-averaged molar mass (M_w) and the *z*-averaged root-mean-square radius of gyration ($\langle R_g \rangle$) of PNIPAM fraction characterized by static light scattering are 4.5×10^5 g/mol and 26.6 nm, respectively. The polydispersity index (M_w/M_n) is ∼1.4 estimated from the relative width $\mu_2/\langle\Gamma\rangle^2$ of the line-width distribution measured in dynamic LLS by using $M_w/M_n \sim 1 + 4\mu_2/\langle\Gamma\rangle^2$.

Differential Scanning Calorimetry Measurements. Phase transitions of the poly(*N*-isopropylacrylamide) (PNIPAM) aqueous solutions with different urea concentrations were characterized with a high sensitivity differential scanning calorimetry (nano-DSC model, TA Instruments) in the temperature range from 5 to 60 °C. A constant pressure of 3.04×10^5 Pa was maintained by a built-in high-pressure piston driven by a computer-controlled linear actuator during all DSC experiments to prevent evaporation of the sample solution. The PNIPAM aqueous solutions with a PNIPAM concentration of 1.0 mg/mL and different urea concentrations of 0, 0.8, 1.5, 3.0, 4.5, or 6.0 M, and the aqueous solutions with the same urea concentration but without PNIPAM as the reference in two capillary cells, were degassed at room temperature for 20 min. DSC measurements were carried out by the following three different methods: (1) Each sample was heated from 5 to 60 °C at a heating rate of 1.00 °C/min. Subsequently the sample was cooled from 60 to 5 °C. The DSC scans were repeated several times to check reproducibility. (2) The processes are the same as those of method 1 except that each sample was maintained isothermally at 5 °C for 30 min in each cycle. (3) Each sample was heated from 5 to 60 °C at a heating rate of 1.00 °C/min and maintained isothermally at 60 °C for 24 h. Then the sample was cooled from 60 to 5 °C. The subsequent processes are the same as method 1. Baselines were collected at a scanning rate of 1.00 °C/min at the same temperature range using the urea aqueous solution with the same concentration in both reference and sample cells. Data acquisition and analysis were performed using NanoAnalyze software provided by TA Instruments. The transition temperature (T_t) was determined as the maximum of an endothermic peak of the heating thermogram or an exothermic peak of the cooling thermogram. The enthalpy change ($\Delta_t H$) during the transition was calculated by integration of the peak area. The errors for the transition temperature and enthalpy are within ±0.04 °C and ±0.7 J g^{-1}, respectively.

RESULTS AND DISCUSSION

In this study, a narrow-distribute PNIPAM sample with weight-average molar mass (M_w) of 4.5×10^5 g/mol and $M_w/M_n \sim 1.4$ was used to reduce the effect of M_w on the phase transition of PNIPAM as it was found that the transition temperature (T_t) of PNIPAM measured by DSC is scaled to the degree of polymerization (N) as $T_t \propto N^{-1/2}$.[44] Figure 1 shows that

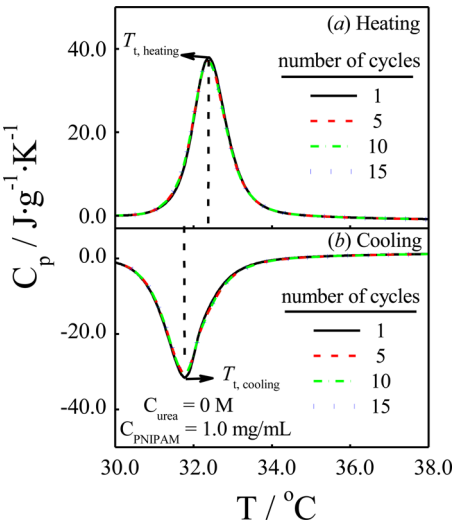

Figure 1. Differential scanning calorimetry (DSC) thermograms of PNIPAM aqueous solution without the addition of urea at four different heating−cooling cycles (cycles 1, 5, 10, 15), where both the heating and cooling rates were 1.00 °C/min and the PNIPAM concentration was 1.0 mg/mL.

without the addition of urea the phase transition of PNIPAM in both heating and cooling processes are reproducible, where the

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PNIPAM concentration was 1.0 mg/mL and the scanning rate was 1.00 °C/min. The arrows in Figure 1 show how the $T_{t,\,heating}$ and $T_{t,\,cooling}$ are defined in our DSC experiments. $T_{t,\,cooling}$ is 31.76 °C which is ~0.6 °C lower than $T_{t,\,heating}$, indicating a hysteresis. This phenomenon is presumably due to the formation of intra and interchain hydrogen bonds in the collapsed state of PNIPAM chains at temperatures higher than its lower critical solution temperature (LCST, ~ 32 °C), which has been observed by different techniques, such as laser light scattering,[13,56] infrared spectroscopy[30,33] and DSC.[29]

Figure 2 shows the temperature dependence of the specific heat capacity (C_p) of PNIPAM aqueous solution containing

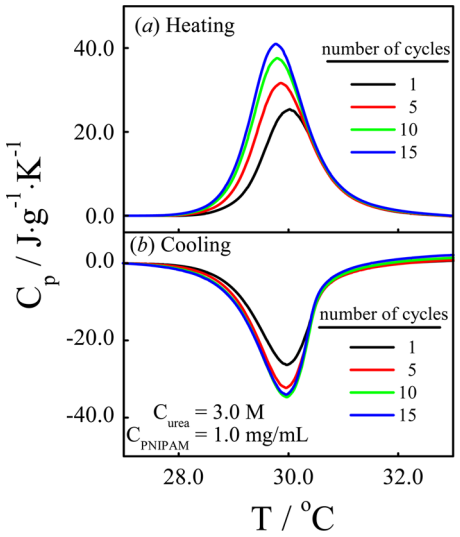

Figure 2. Differential scanning calorimetry (DSC) thermograms of PNIPAM aqueous solution with the addition of urea at four different heating−cooling cycles (cycles 1, 5, 10, 15), where both the heating and cooling rates were 1.00 °C/min, and the PNIPAM concentration (C_{PNIPAM}) and urea concentration (C_{urea}) were 1.0 mg/mL and 3.0 M, respectively.

3.0 M urea at four different heating−cooling cycles (circles 1, 5, 10, 15). The DSC experiments in Figure 2 were carried out by method 1, i.e., the PNIPAM aqueous solution and the reference with the same concentration of urea were maintained isothermally neither at 5 °C nor at 60 °C. Note that small amounts of possible product of hydrolysis of urea ammonium carbonate did not have noticeable effect on the phase transition of PNIPAM due to the slow hydrolysis rate of urea at 60 °C in aqueous solution.[57] During the heating and cooling processes, the enthalpy change of the phase transition which was calculated by integration of peak area increases with the number of DSC cycles. $T_{t,\,heating}$ decreases with the number of cycles and $T_{t,\,cooling}$ is nearly a constant. Moreover, the enthalpy change of phase transition of PNIPAM aqueous solution containing 3.0 M urea during the first heating process ($\Delta_tH_{heating}$) is 38.1 J/g, which is smaller than the value of $\Delta_tH_{heating}$ (47.4 J/g) for the phase transition of PNIPAM solution without the addition of urea. Furthermore, the absolute value of $\Delta_tH_{heating}$ and $\Delta_tH_{cooling}$ increases with the number of DSC cycles, which we will discuss in detail in the following sections.

Figure 3 shows number of DSC cycles dependence of the enthalpy change ($\Delta_tH_{heating}$) during the phase transition of PNIPAM solutions with different concentrations of urea. As can be seen, $\Delta_tH_{heating}$ decreases as the concentration of urea

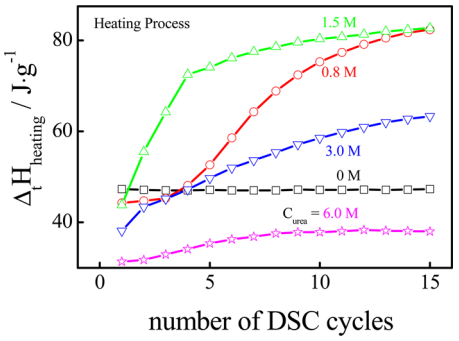

Figure 3. Number of DSC cycles dependence of enthalpy change ($\Delta_tH_{heating}$) during the phase transition of PNIPAM solutions with different urea concentrations in the heating processes, where both the heating and cooling rates were 1.00 °C/min and the PNIPAM concentration was 1.0 mg/mL.

(C_{urea}) increases from 0 to 6.0 M during the first heating process. It was suggested by Shibayama et al.[40,42] and Ebara et al.[58] that the dissociation of water molecules around the hydrophobic groups of the PNIPAM chains leads to the phase transition of PNIPAM in aqueous solution at high temperature. Thus, the number of water molecules dehydrated from each NIPAM repeating unit[59,60] could affect the endothermic heat during the phase transition. Meanwhile, Kuharski et al.[61] reported that one urea molecule could displace several water molecules from the solvation shell around an apolar solute, and Stumpe et al.[53] found that urea interacts preferentially with apolar residues using comprehensive molecular dynamics simulations of 22 tripeptides. With the addition of urea the number of hydrated water molecules around the isopropyl group decreases,[62] and the corresponding $\Delta_tH_{heating}$ decreases.

For a given urea concentration, $\Delta_tH_{heating}$ increases with the number of DSC cycles and then levels off after ~15 cycles. Grinberg et al. investigated the phase transition of PNIPAM hydrogels by high-sensitivity differential scanning calorimetry and calculated the heat from the dehydration of apolar groups, polar groups, and interactions of residues (van der Waals and/or hydrogen bonding).[27] The enthalpies of the three processes are 78, 402, and −441 J g^{-1}, respectively. Recently, Cho et al. suggested that the phase transition of PNIPAM aqueous solution comprises at least two processes by using temperature modulated differential scanning calorimetry (TMDSC) and the single endothermic peak could be separated into an endothermic and exothermic peak.[28] However, the authors stated that the exact value of the transition heat for each process could not be precisely obtained because the frequency region which TMDSC can cover is limited (10^{-1}−10^{-2} Hz). For simplicity, we assign the endothermic and exothermic peaks to the heat related to the apolar group (Δ_tH_a) and that related to the polar group (Δ_tH_p), respectively; i.e., Δ_tH_a is contributed by the rearrangement of water molecules around hydrophobic groups and the van der Waals interaction between these groups, and Δ_tH_p is related to the dehydration of hydrophilic groups (amide groups) and the formation of inter and/or intra hydrogen bonds and/or van der Waals interactions of these groups. By Fourier transform infrared spectroscopy (FTIR), Sagle et al. reported that the fraction of urea bound to the amide groups of PNIPAM chains is negligible at the temperature lower than LCST when the urea concentration (C_{urea}) is below ~1 M, and the fraction increases linearly with the urea concentration when C_{urea} is above this value.[55] Our previous

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results from laser light scattering experiments showed that no aggregation can be observed for collapsed PNIPAM globules when the C_{urea} is below a critical urea concentration ~1.2 M when the temperature is higher than the lower critical solution temperature (LCST).[14] Thus, the fraction of urea bound to amide groups of PNIPAM chains is around zero when C_{urea} is 0.8 M in our study. Assuming that urea molecules displace water molecules forming hydrogen bonds with amide groups at the temperature higher than LCST and the recovery needs at least several hours to complete when the temperature decreases below LCST, the absolute value of Δ_tH_p decreases to around 0 after ~15 cycles and Δ_tH_a is nearly constant; therefore, the total $\Delta_tH_{heating}$ increases with the number of DSC cycles. Note that during the heating process of the 15th cycle the $\Delta_tH_{heating}$ is 82.1 J g^{-1}, which is close to the value of heat from the dehydration of apolar groups (78 J g^{-1}) reported by Grinberg et al.[27] Moreover, the change of $\Delta_tH_{heating}$ between the last cycle and the first cycle increases as C_{urea} changes from 0 to 1.5 M and then decreases as C_{urea} changes from 1.5 to 6.0 M, indicating that more water molecules bound to the amide groups of PNIPAM chains have been replaced by urea molecules at the temperature lower than LCST when C_{urea} is higher than ~1 M.

As shown in Figure 4, in the cooling processes, the enthalpy change ($\Delta_tH_{cooling}$) is nearly constant at different DSC cycles

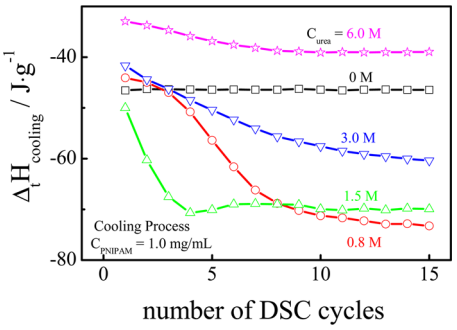

Figure 4. Number of DSC cycles dependence of enthalpy change ($\Delta_tH_{cooling}$) during the phase transition of PNIPAM solutions with different urea concentrations in the cooling processes, where both the heating and cooling rates were 1.00 °C/min and the PNIPAM concentration was 1.0 mg/mL.

when C_{urea} = 0 M. When C_{urea} is higher than 0 M, the absolute value of $\Delta_tH_{cooling}$ increases at the first several DSC cycles and levels off at about the 15th DSC cycle. Similar to what we have mentioned above, $\Delta_tH_{cooling}$ also contains the heat related to the apolar groups (Δ_tH_a') and that related to the polar groups (Δ_tH_p'). Note that Δ_tH_a' is exothermic and Δ_tH_p' is endothermic. For a given certain C_{urea}, Δ_tH_a' is nearly constant and Δ_tH_p' decreases to ~0 after several DSC cycles because of the displacement of water molecules bound to amide groups by urea molecules at the temperature above LCST, which leads to the increase in the absolute value of $\Delta_tH_{cooling}$.

Figure 5 shows the effect of number of DSC cycles on the $T_{t,heating}$ and $T_{t,cooling}$ of PNIPAM solution with different concentrations of urea. When C_{urea} is 0 M, both $T_{t,heating}$ and $T_{t,cooling}$ are independent of the number of DSC cycles. Both $T_{t,heating}$ and $T_{t,cooling}$ at the first heating cycle decrease with C_{urea}, indicating that urea molecules promote the formation of the collapsed and aggregated state of PNIPAM presumably due to the reason that urea molecules interact with PNIPAM in a

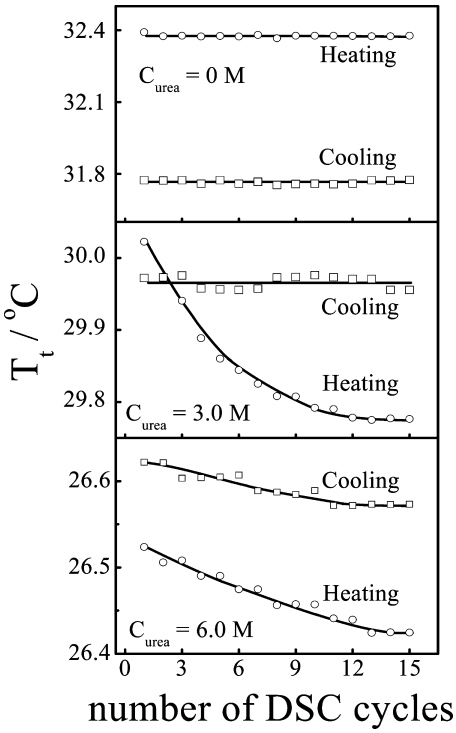

Figure 5. Effect of the number of DSC cycles on the transition temperature (T_t) of PNIPAM aqueous solutions with different concentrations of urea, where the PNIPAM concentration (C_{PNIPAM}) was 1.0 mg/mL and urea concentrations (C_{urea}) were 0, 3.0, and 6.0 M, respectively.

bivalent manner as cross-linkers of PNIPAM and facilitate the hydrophobic collapse of PNIPAM chains.[14,55] When C_{urea} is 3.0 M, $T_{t,heating}$ decreases with the number of DSC cycles, indicating that more and more urea molecules have replaced the water molecules bound to the amide groups of PNIPAM. However, $T_{t,cooling}$ is nearly constant at different numbers of DSC cycles presumably due to the reason that the segmental motion of the PNIPAM chains is restricted when the temperature is higher than the LCST[63] and it is difficult for one urea molecule which already forms a hydrogen bond with an amide group of PNIPAM to form another hydrogen bond with another amide group in the same or different PNIPAM chains. Thereby, $T_{t,heating}$ becomes smaller than $T_{t,cooling}$ from the third DSC cycle at the urea concentration of 3.0 M. When C_{urea} is 6.0 M, $T_{t,heating}$ decreases with the number of DSC cycles, and it is smaller than $T_{t,cooling}$ at all DSC cycles.

Figure 6 shows that the C_{urea} dependence of $\Delta_tH_{heating}$ at four different cycles in the heating processes. During the first heating process, $\Delta_tH_{heating}$ decreases linearly with C_{urea}, indicating that more and more water molecules around the apolar isopropyl groups are displaced by urea molecules. The C_{urea} dependence of $\Delta_tH_{heating}$ changes is more complicated for the following DSC cycles. For example, in the tenth cycle, $\Delta_tH_{heating}$ increases to a maximum when C_{urea} changes from 0 to 1.5 M and then decreases when C_{urea} changes from 1.5 to 6.0 M. The increase in $\Delta_tH_{heating}$ is presumably due to the decrease in the absolute value of Δ_tH_p because of the displacement of water molecules bound to amide groups at the temperature higher than LCST, as mentioned before. Because of the displacement of water molecules bound to amide groups by urea molecules at the temperature lower than LCST when C_{urea} is higher than ~1 M,[55] the effect of Δ_tH_p on the $\Delta_tH_{heating}$ decreases with the C_{urea}.

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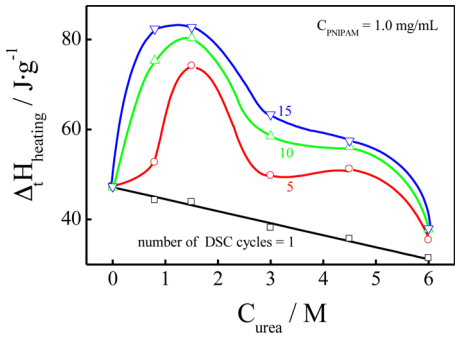

Figure 6. Urea concentration dependence of the enthalpy changes ($\Delta_t H_{heating}$) of PNIPAM aqueous solutions at four different heating processes (cycles 1, 5, 10, and 15) in the heating processes, where both the heating and cooling rates were 1.00 °C/min and the PNIPAM concentration (C_{PNIPAM}) was 1.0 mg/mL.

As mentioned above, after ~15 cycles almost all water molecules bound to amide groups of PNIPAM have been displaced by urea molecules when DSC experiments were carried out by method 1, i.e., without being maintained isothermally at 5 or 60 °C. One can imagine that if the PNIPAM solution was maintained for 24 h at the temperature higher than LCST, more water molecules would be displaced during the incubation in one cycle, and more urea molecules bound to amide groups at high temperature would also be displaced by water molecules if the PNIPAM solution was maintained at low temperature (5 °C) for longer time because the displacement might be kinetically controlled. Figure 7

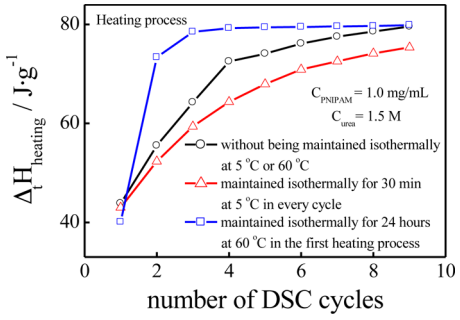

Figure 7. Number of DSC cycles dependence of enthalpy change ($\Delta_t H_{heating}$) during the phase transition of PNIPAM solutions in the heating processes by different DSC methods, as mentioned in the Experimental Section: (○) method 1, without being maintained isothermally at 5 or 60 °C; (△) method 2, maintained isothermally for 30 min at 5 °C in each cycle; (□) method 3, maintained isothermally for 24 h at 60 °C in the first heating process, where both the heating and cooling rates were 1.00 °C/min and the PNIPAM concentration (C_{PNIPAM}) and urea concentration (C_{urea}) were 1.0 mg/mL and 1.5 M, respectively.

shows that the $\Delta_t H_{heating}$ increases in the first three cycles and levels off after the third cycle, indicating that incubation at the temperature higher than LCST facilitates the displacement of water molecules bound to amide groups by urea molecules. Figure 7 also shows that when the PNIPAM solution was maintained isothermally for 30 min at 5 °C in each cycle, $\Delta_t H_{heating}$ increases slowly with the number of DSC cycles, indicating that incubation at 5 °C facilitates the replacement of urea molecules by water molecules. Our studies also revealed that when the PNIPAM solution was kept in the refrigerator for ~10 days after 15 heating–cooling cycles, $\Delta_t H_{heating}$ is 42.7 J g^{-1} which is close to the original value (43.0 J g^{-1}) as if without any treatment, indicating that all urea molecules bound to amide groups at the temperature higher than LCST have been replaced by water molecules during the incubation at 5 °C. Figure 8 shows the schematic diagram of the effect of urea on the phase transition of PNIPAM solutions.

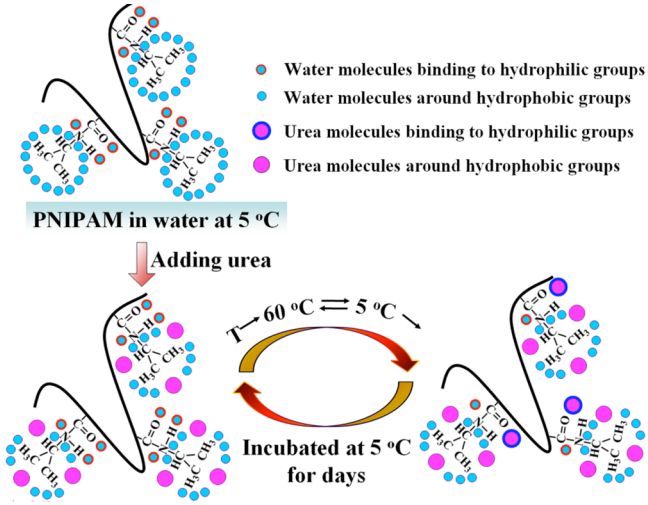

Figure 8. Schematic diagram of the effect of urea on the phase transition of PNIPAM solutions.

CONCLUSION

In conclusion, we have investigated the effect of urea on the phase transition of PNIPAM solutions by DSC. When C_{urea} is 0 M, the phase transition of PNIPAM solutions is reproducible. When C_{urea} is higher than 0 M, the enthalpy change ($\Delta_t H_{heating}$) containing the endothermic heat ($\Delta_t H_a$) related to the apolar isopropyl groups and the exothermic heat ($\Delta_t H_p$) related to the polar amide groups in heating processes increase with the number of DSC cycles, indicating that the absolute value of $\Delta_t H_p$ decreases to ~0, presumably due to the displacement of water molecules bound to amide groups of PNIPAM by urea molecules at the temperature higher than LCST of PNIPAM. Moreover, during the first heating process $\Delta_t H_{heating}$ decreases with the C_{urea} because more water molecules around the isopropyl groups are displaced by urea molecules with C_{urea}, and thus, $\Delta_t H_a$ decreases. Furthermore, the urea molecules bound to the amide groups at the temperature higher than LCST can be displaced slowly by water molecules at 5 °C.

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Notes

The authors declare no competing financial interest.

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