Microcalorimetric Investigation on Aggregation and Dissolution of Poly(*N*-isopropylacrylamide) Chains in Water

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ABSTRACT: Aggregation and dissolution of poly(*N*-isopropylacrylamide) (PNIPAM) in water were investigated using an ultrasensitive differential scanning calorimetry (US-DSC) and a pressure perturbation calorimetry (PPC). US-DSC reveals that both the aggregation and dissolution of PNIPAM chains are greatly dependent on the scanning rate, indicating that the processes are kinetically controlled. The hysteresis in the dissolution process was found to have a nonequilibrium nature, which is thought to be related to the additional hydrogen bondings formed in the collapsed state of PNIPAM chains. A bimodal appearing in the cooling process at a slow scanning rate indicates the dissolution involves two different processes, i.e., the disruption of additional hydrogen bondings and the dissolution of the collapsed chains. PPC reveals that the solvent accessible surface area of PNIPAM chains in the cooling process is smaller than that in the heating process, which further indicates the dissolution of the PNIPAM aggregates involves such two processes.

Introduction

Protein folding as a remaining challenge has recently gained more attention. Protein chains can normally fold into their defined tertiary structure via a specific pathway.[1,2] On the other hand, it was also found that protein chains may misfold to form aggregates leading to some diseases.[3] Considering the important macromolecular nature of protein chains with a complex structure, it is helpful to simplify the problem by using a synthetic homopolymer as a model system to study the folding and aggregation.

A long time ago, Stockmayer[4] suggested that a flexible homopolymer chain can transit its conformation from an expanded coil to a collapsed globule on the basis of Flory's mean-field theory.[5] Such a coil-to-globule transition has been both investigated theoretically[6−13] and experimentally.[14−26] Among the homopolymers studied, poly(*N*-isopropylacrylamide) (PNIPAM) with a lower critical solution temperature (LCST) at ∼32 °C in aqueous solution has been extensively used since its conformational change can be conveniently examined by adjusting the solvent quality via temperature.[21−27] As shown in many past studies, the interchain aggregation often occurs before individual chains reach its fully collapsed globule state. Therefore, it is difficult to conduct such a coil-to-globule transition in a true single chain fashion. In some earlier works,[21−23] a small amount of sodium dodecyl sulfate (SDS) was added to prevent the interchain aggregation of PNIPAM in water. The introduction of SDS probably alternates the nature of PNIPAM chains since SDS can form complex with PNIPAM.[22−24] In an extremely dilute solution, Wu et al.[26] directly observed the coil-to-globule and globule-to-coil transitions of PNIPAM in water without any surfactant. They also found a hysteresis in the globule-to-coil transition process, which was attributed to the formation of some additional hydrogen bondings in the collapsed state, but it is not conclusive.

The study of aggregation and dissolution of synthetic homopolymer chains might shed some light on the misfolding and unfolding of protein chains. To our knowledge, there have been only a few investigations on the dissolution of polymer chain aggregates because the aggregation usually leads to precipitation, which makes the characterization difficult and nonreproducible.

On the other hand, ultrasensitive microcalorimetry (US-DSC) can measure an energy change as small as 0.02 μcal/s involved in a dynamic process of a macromolecular chain in a dilute solution,[28−30] whereas pressure perturbation calorimetry (PPC) can precisely determine the coefficient of thermal volume expansion of macromolecules in solution.[31,32] Therefore, the information about the solvent-induced accessible surface area on which the solute interacts with solvent can be obtained. Previous studies showed that the aggregation of PNIPAM chains in a dilute solution at higher temperatures does not lead to precipitation even in the absence of surfactant.[28−38] Therefore, the aggregation should have no effect on the measurements of US-DSC and PPC. This is why we are able to study the aggregation and dissolution of PNIPAM chains in dilute aqueous solution. The present work can help us to have a better understanding of phase transition of PNIPAM chains in water.

Experimental Section

Sample Preparation. *N*-Isopropylacrylamide (NIPAM) from Eastman Kodak was recrystallized three times in a benzene/*n*-hexane mixture. PNIPAM was synthesized by radical polymerization in benzene with azobis(isobutyronitrile) (AIBN) as the initiator; the details can be found elsewhere.[39] The resultant PNIPAM was fractioned in a acetone/hexane mixture. The molecular weight (M_w) determined by static laser light scattering is 1.6×10^6, and the polydispersity index (M_w/M_n) is ∼1.5 estimated from the line width in dynamic laser light scattering.[40]

US-DSC measurements were carried on a VP DSC from MicroCal. The volume of the sample cell was 0.509 mL. The reference cell was filled with deionized water. PNIPAM

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solution with a concentration of 1.0×10^{-3} g/mL was degassed at 25 °C for half an hour. The solution was equilibrated at 10 °C for 2 h before the heating process. In the cooling process, the sample solution was equilibrated at 80 °C for 2 h to eliminate the effect of thermal history. The transition temperature (T_m) was taken as that centered at the transition. The enthalpy change (ΔH) during the transition was calculated from the area under each peak.

PPC measurements were performed on a VP-DSC with a pressure perturbation accessory. The applied pressure during the compression cycle was about 5 bar. The reference and sample cells have an identical volume (0.509 mL) and open to a common pressure chamber containing a sensor. The theory for PPC was detailed elsewhere.[31] Using PPC, we are able to measure the heat change (ΔQ) between a PNIPAM solution in the sample cell and water in the reference cell, which is induced by a pressure change (Δp) at a certain temperature. The coefficient of thermal expansion (α_p) of PNIPAM was calculated as

$$\alpha_p = \alpha_w - \frac{\Delta Q}{T v_p m_p \Delta p} \quad (1)$$

where α_p and α_w are respectively the thermal expansion coefficient of PNIPAM and water and v_p and m_p are respectively the partial specific volume and mass of PNIPAM in water. The relative volume changes ($\Delta V/V$) in the phase transition and in the temperature range of T_0 to T_e can be obtained by

$$\frac{\Delta V}{V} = \int_{T_0}^{T_e} \alpha_p \, dT \quad (2)$$

The density of PNIPAM in water was measured on a DMA 4500 densimeter from Anton-Parr (Austria) with precision less than $\pm 5 \times 10^{-5}$ g/cm^3. The concentration of each solution was varied over the range of 0.05−1.00 mg/mL. The measurements were calibrated with air and deionized water as two standards. The partial specific volume of PNIPAM (v_p) was determined as a sum of the intercept and the slope of the inverse density of solution versus concentration of PNIPAM.[29]

Results and Discussion

First, we examined the effect of aggregation because it could greatly influence a calorimetric measurement, reflecting in a sharp drop of the heat capacity after the heat absorption peak.[21] In all of the experiments here, we did not observe such a phenomenon, and all the data were reproducible, indicating that the aggregation does not have a noticeable effect on the measurements. This is because the association of linear PNIPAM chains in a dilute solution leads to stable aggregates since the collapsed chains still contain more than 70% of water in its hydrodynamic volume even in the fully collapsed state.[26] Such a negligible effect of the aggregation on the microcalorimetric measurements of PNIPAM in water was revealed before.[29,34]

Figure 1 shows the temperature dependence of the specific heat capacity (C_p) in one heating-and-cooling cycle, where both the heating and cooling rates were 1.00 °C/min. It can be seen that linear PNIPAM chains have a smaller C_p at the temperatures higher than its LCST in either the heating or cooling process. It is known that the denaturation of a protein is accompanied by an increase of C_p, which is attributed to the exposure of nonpolar groups to water or an increase of polymer−water contacts.[41] The decrease of C_p at an elevated temperature implies that linear PNIPAM coils collapse into a more compact structure.[21] Note that both intrachain collapse and interchain aggregation occur during the coil-to-globule transition because the solution

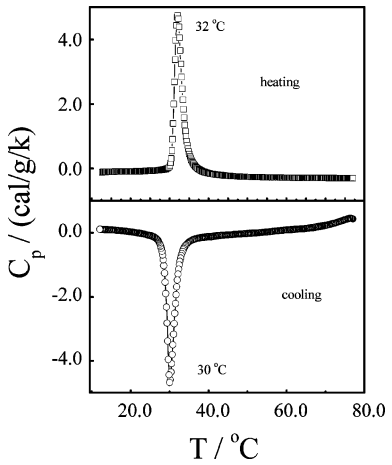

Figure 1. Temperature dependence of the specific heat capacity (C_p) of PNIPAM in the aggregation and dissolution processes in water, where the heating and cooling rates were 1.00 and 0.992 °C/min, respectively.

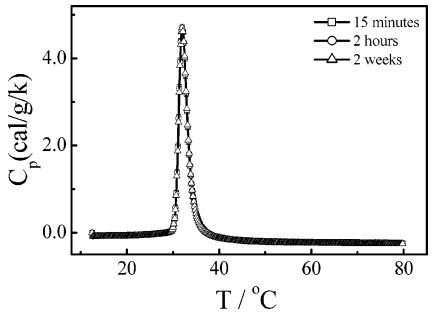

Figure 2. Incubation time dependence of the specific heat capacity (C_p) of PNIPAM in water in the heating process, where the heating rate was 1.00 °C/min, and the sample was incubated at 10 °C before reheating.

used for US-DSC measurements is not as dilute as that in light scattering.[26]

The transition temperature (T_m) in the cooling process ~2.0 °C lower than that in the heating process clearly indicates a hysteresis. A similar phenomenon was previously observed in the folding and unfolding of a single PNIPAM chain in water,[26] which was attributed to some additional intrachain hydrogen bondings among PNIPAM chains formed in the collapse state at high temperatures. Obviously, such an additional hydrogen bonding is not a unique feature for a single PNIPAM chain but also for the collapsed PNIPAM chains in the aggregates. From the area of each exothermic or endothermic peak in Figure 1, we determined the enthalpy change (ΔH) in the transition. The enthalpy change in the cooling process is ~30% less than that in the heating process, indicating that the aggregated chains only partially dissolved during the transition in cooling process. The aggregation can be completely removed by incubating the solution at 10 °C for a short time of 15 min (Figure 2). The fact indicates that the hysteresis is due to some additional hydrogen bondings formed in the collapsed state.

Figure 3 shows the temperature dependence of specific heat capacity (C_p) at different heating rates. As the heating rate increases from 0.081 to 1.492 °C/min, the US-DSC curves become more asymmetrical with the transition temperature (T_m) shifting from 31.5 to 32.5 °C. This is because intrachain contraction and interchain association compete with each other in the heating process. The slow heating allows linear PNIPAM chains

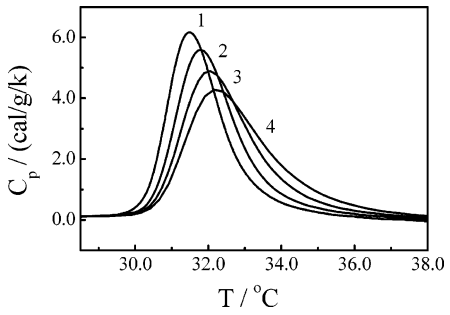

Figure 3. Effects of heating rate on the aggregation of PNIPAM chains in water, where the heating rate was (1) 0.081, (2) 0.495, (3) 1.00, and (4) 1.49 °C/min.

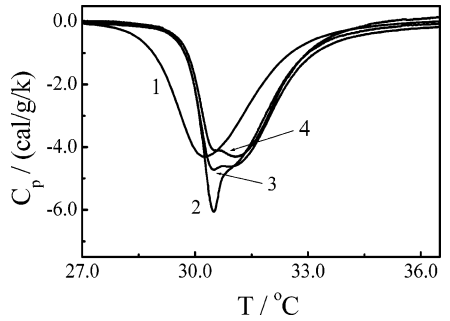

Figure 4. Effects of cooling rate on the dissolution of PNIPAM chains in water, where the cooling rate was (1) 0.825, (2) 0.078, (3) 0.062, and (4) 0.039 °C/min.

to collapse and aggregate occur simultaneously, while the faster heating leads to the collapse of individual chains before and after the interchain aggregation. In other words, the formed structure is kinetically controlled.

Figure 4 shows the temperature dependence of specific heat capacity (C_p) of the PNIPAM aggregates at different cooling rates. As the cooling rate decreases from 0.825 to 0.039 °C/min, the US-DSC curves become much more asymmetrical. Particularly, when the cooling rate is lower than 0.328 °C/min, a bimodal transition is observed with one exothermic peak centered at ~30.5 °C and the other at ~31 °C. The latter becomes dominant as the cooling rate decreases. In our preliminary experiments, such a bimodal transition was never observed in the heating process even at a heating rate as low as 0.030 °C/min. We think the additional hydrogen bondings formed in the collapsed state should be responsible for the behavior. As the temperature decreases, the additional hydrogen bondings are first disrupted, and the aggregates are gradually dissolved from the outer layer to the interior. The peaks located at 31 and 30.5 °C are attributed to disruption of the additional hydrogen bondings and the dissolution of the collapsed chains, respectively. Note that the hysteresis in the dissolution process becomes smaller as the cooling rate decreases. It is expected that there would be no hysteresis when the cooling is infinitely slow. Unfortunately, because of the limitation of the US-DSC, we only observed the decrease of the hysteresis instead of the disappearance.

Figure 5 shows that the transition temperature (T_m) linearly increases with the heating rate, indicating that the intrachain contraction and interchain association of PNIPAM chains in solution are dependent on the heating rate because the chain folding and association cannot follow the temperature change. In contrast, the decrease of T_m with an increasing cooling rate indicates

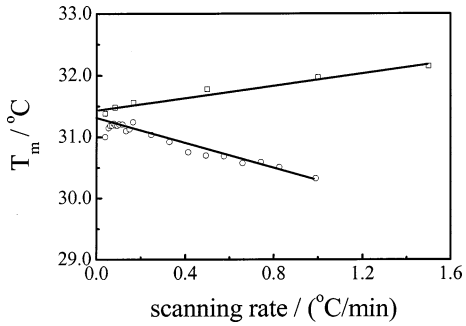

Figure 5. Scanning rate dependence of transition temperature (T_m).

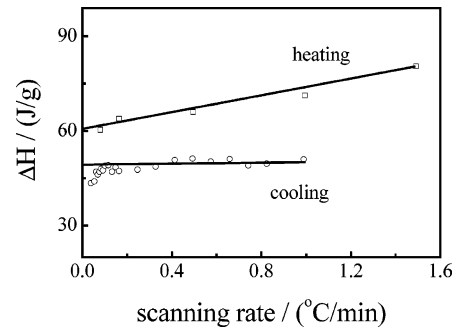

Figure 6. Scanning rate dependence of enthalpy change (ΔH).

the dissolution of the collapsed PNIPAM chains also cannot follow the temperature change. From the extrapolation of T_m in both the heating and cooling processes to the zero scanning rate, we obtained $T_{m,0}$ = 31.4 °C in both the heating and cooling processes. This demonstrates that the hysteresis in the association and dissolution of PNIPAM in water is not an equilibrium phenomenon, but a kinetic effect, which is different from the folding and unfolding of individual PNIPAM chains in an extremely dilute solution.[26] Therefore, the LCST of PNIPAM from a normal DSC or turbidity measurements is not the true LCST on the spinodial line.

Figure 6 shows the scanning rate dependence of the enthalpy change (ΔH) of the transitions. It can be seen that the enthalpy change linearly increases with the heating rate. Grinberg et al.[29] reported that PNIPAM gels exhibit a similar behavior and suggested that the volume change the phase separation might be responsible for the increase in the enthalpy change. Our laser light scattering experiments revealed that a fast heating led to smaller particles; i.e., more intrachain contraction and less interchain association happen, which probably results in more additional hydrogen bondings with a large enthalpy change. At a slow rate, the chains are associated with each other before their collapse, leading to smaller enthalpy change. In the cooling process, the additional hydrogen bondings formed in the collapsed state are disrupted, and the aggregates are dissolved gradually from the outer layer to the core. The enthalpy change related to such a dissolution process is controlled by the cooperative diffusion of the chains in water. That is why it is nearly independent of the cooling rate. This is similar to the enthalpy change observed in the denaturation of some plant proteins.[30]

Another point is that the enthalpy change (ΔH_m) in the cooling process is smaller than that in the heating process. The phenomenon is often found in liquid crystals because of a very slow or broad process in cooling process. In the present study, it indicates that

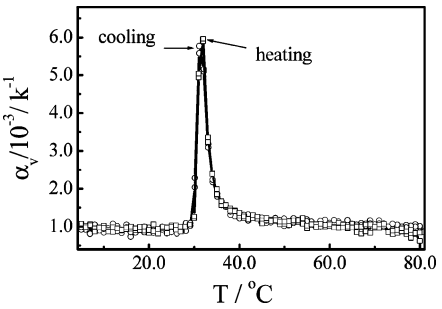

Figure 7. Temperature dependence of the coefficient of thermal expansion (α_p) of PNIPAM in the aggregation and dissolution processes in water, where the average heating and cooling rates were 0.250 and 0.245 °C/min, respectively.

the enthalpy change arises from the phase transition of chains and the formation or disruption of hydrogen bondings. The extrapolation of the enthalpy change to the infinitely slow heating rate yields a value (ΔH_0). Using this value, we estimated that the fraction (f) of dissolved PNIPAM chains during the phase transition is ~80% by using $f = \Delta H_m/\Delta H_0$.

Figure 7 shows the temperature dependence of coefficient of thermal expansion (α_p) in one heating-and-cooling cycle. It is known that the partial volume of a solute includes both its intrinsic volume and the volume change of solvent induced by its interaction with the solute. In other words, the solvation has a great effect on the partial volume and the coefficient of thermal expansion (α_p), especially in aqueous solution due to the unusual properties of water. It is known that hydrophobic moieties acting as some structure makers usually show a smaller α_p at lower temperatures with a larger positive temperature coefficient. On the other hand, hydrophilic groups as some structure breakers exhibit a larger positive α_p with a larger negative temperature coefficient.[32] Figure 7 shows that the transition has a large positive α_p, indicating that linear PNIPAM chains change from hydrophilic into hydrophobic during the coil-to-globule transition. The relative volume change ($\Delta V/V$) at the transition in the heating process is 1.54% on the basis of eq 2, ~10% larger than that in the cooling process. The smaller solvent accessible surface area (ASA) in the cooling process suggests that linear PNIPAM chains are less exposed to water in the cooling process. As discussed above, the dissolution of the collapsed PNIPAM aggregates involves the disruption of the additional hydrogen bonding formed in the collapse state and dissolution of the chains inside the aggregates. That is why the surface area of the chains exposed to water at the LCST is smaller. Figure 7 also shows that the apparent LCST values obtained from PPC in the heating and cooling processes are some different from those from the US-DSC. The hysteresis observed in the PPC experiments is smaller. This is understandable since PNIPAM solution was cooled at a slow rate of 0.245 °C/min. On the other hand, the pressure applied to the solution might be favorable to the dissolution of PNIPAM chains.

Conclusions

The effects of heating and cooling rates on the aggregation and dissolution of linear poly(*N*-isopropylacrylamide) chains in water were investigated by an ultrasensitive differential scanning calorimetry and a pressure perturbation calorimetry. In both the heating and cooling processes, PNIPAM shows a phase transition with a lower critical solution temperature (LCST) of ~31−32 °C. The LCST observed in the cooling process is slightly lower than that in the heating process. Such a hysteresis is understandable because the apparent enthalpy change in US-DSC is obtained in a kinetic fashion. The fact that US-DSC curves in the slow cooling processes containing two peaks indicates that dissolution of the chain aggregates involves two processes, presumably, the disruption of additional hydrogen bondings formed in the collapsed state and the dissolution of the collapsed and entangled chains. The smaller solvent accessible surface area in the cooling process obtained in pressure perturbation calorimetry further supports that the dissolution of the collapsed and entangled PNIPAM chains undergoes the above two processes.

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