Thermodynamically Stable Globule State of a Single Poly(*N*-isopropylacrylamide) Chain in Water

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Three decades ago Stockmayer[1] first suggested that a flexible polymer chain can transit its conformation from an expanded coil to a collapsed globule on the basis of Flory's mean-field theory.[2] Since his prediction, theoretical and experimental studies of this coil-to-globule transition have been extensively conducted.[3-12] The majority of the reported works were based on polystyrene in various organic solvents because of two very special requirements for the studied polymer chain; namely, its molecular weight should be as high as possible and its molecular mass distribution should be as narrow as possible. Many useful experimental results have been obtained by using improved modern equipment,[11,12] especially laser light scattering (LLS) which includes static and dynamic LLS, interpreted by a well-developed theory.[8,9]

Grosberg[13] *et al.* in one of their recent papers stated that, on the basis of their quantitative theories of coil-to-globule transition,[8] a true equilibrium single-chain collapse has not been experimentally observed yet for simple uncharged homopolymers without mesogenic groups. They predict a two-stage kinetics of a single-chain collapse process, namely, a fast crumpling process of unknotted polymer chain followed by a slow knotting process of the collapsed polymer chain. This two-stage kinetics has recently been observed by Chu[14,15] *et al.* in a kinetic study of a single polystyrene chain collapse process before its precipitation. In their marvelous experiment, dynamic laser light scattering (LLS) was employed to monitor the change of the hydrodynamic radius (R_h) of a single polystyrene chain in cyclohexane after an abrupt temperature jump from 35 to 29 °C. Their measured hydrodynamic radius distribution showed two quite different species which were attributed to the collapsed single polystyrene chain and the aggregate of the collapsed polystyrene chains. From the change of R_h as a function of time after the temperature jump, they reported, for the first time, two relaxation times τ_{crum}, for the crumpled globule state, and τ_{eq}, for the compact globule state.

The great interest in this coil-to-globule transition is not only due to its importance as a general and fundamental concept in polymer physics and solution dynamics but also due to its deep implications in many biological systems, such as protein folding[16] and DNA packing.[17] Therefore, in this aspect, the investigation of the coil-to-globule transition of some water-soluble polymers in aqueous solution should be even more interesting. However, this is even more difficult and challenging due to the two above-mentioned sample requirements. So far, only a limited number of experimental works have been reported.[18-20] Among them, Kubota[20] *et al.* first seriously studied the coil-to-globule transition of a single poly(*N*-isopropylacrylamide) (PNIPAM) chain in water. With some effort, they only got three PNIPAM samples with $M_w > 4.10 \times 10^6$ and $M_w/M_n > 1.3$. This is why they only observed a limited chain collapse before the system reached a thermody-

namically unstable regime, i.e., the phase separation and aggregation.

It is known that the swollen PNIPAM gel has a sharp volume phase transition at its lower critical solution temperature (LCST, ~31 °C) when the temperature is raised from ~25 to ~35 °C and the volume change can be as high as 100 times.[21] On the one hand, this sharp and large volume change implies that it should be easier to observe a true globule state of a single polymer chain if PNIPAM is used; on the other hand, considering the driving force for the phase transition, the hydrophobic and hydrophilic interactions in water should be much stronger than the van de Waals interactions among polymer segments in organic solvents, so that it should be easier for a single PNIPAM chain to reach a collapsed state in water than for polystyrene in organic solvents. In this paper, we show that with a carefully prepared PNIPAM system this higher-degree collapsing of a single polymer chain in water is experimentally realized for the first time, to our knowledge; that is, a thermodynamically stable globule state of a single polymer chain is obtained.

On the basis of our previous experience,[22] a combination of both fractionation and filtration enabled us to successfully prepare an extremely dilute aqueous solution (C ~5 μg/mL) with a very narrowly distributed high molecular weight PNIPAM sample ($M_w = 1.08 \times 10^7$ and $M_w/M_n < 1.06$). In this study, both the radius of gyration (R_g) from static LLS and the hydrodynamic radius (R_h) from dynamic LLS were measured as a function of the solution temperature around the Flory Θ−temperature (30.59 °C). The process and state of collapse were respectively monitored in terms of the radius of R_g (and R_h) and the ratio of R_g/R_h.

A commercial LLS spectrometer (ALV/SP-150, Germany) equipped with an ALV-5000 multi-τ digital correlator was used with a solid-state laser (ADLAS DPY425II, Germany; output power ≈ 400 mW at $\lambda = 532$ nm) as the light source. The incident light beam was vertically polarized with respect to the scattering plane, and the laser light intensity was regulated with a beam attenuator (Newport M-925B) so that possible localized heating in the light-scattering cuvette can be avoided. In our present setup, the coherent factor β in dynamic LLS is ~0.87, a rather high value for an LLS spectrometer capable of doing both static and dynamic LLS simultaneously. This is why we were able to carry out dynamic LLS in such an extremely dilute solution with a reasonable signal-to-noise ratio. With proper modifications,[23] our LLS spectrometer is now capable of doing both static and dynamic LLS continuously in the scattering angle range of 6−154°. This accessible small-angle range is very useful in the measurement of high molecular weight polymer chains because, on the one hand, for an accurate determination of R_g, the condition of $R_g q < 1$ is required, where q [=(4$\pi n/\lambda_0$) (sin(θ/2)], with n, λ_0, and θ being the solvent refractive index, the laser light wavelength in vacuum, and the scattering angle, respectively, and, on the other hand, the extrapolation to the zero scattering angle and the interference of the internal motions associated with the long polymer chain can be avoided. In addition, this accessible small-angle range has enabled us to study an extremely dilute solution because for a long polymer chain the scattered light intensity at small scattering angle is much stronger than that at high scattering angle.
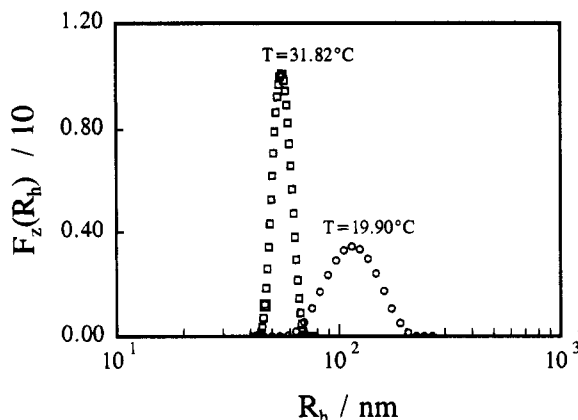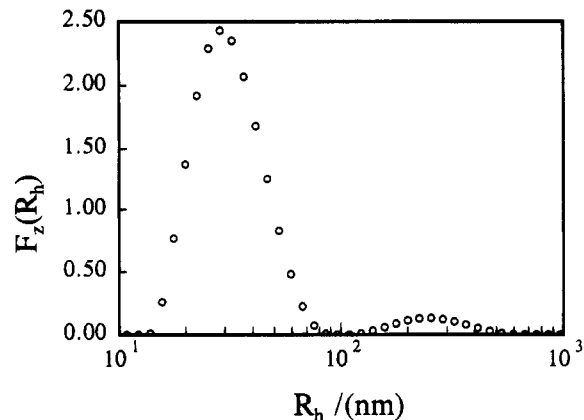
Figure 1. Typical hydrodynamic radius distributions $f(R_h)$ of the dilute PNIPAM aqueous solution ($C = 4.66 \times 10^{-6}$ g/mL) under good solvent conditions (O, $T = 19.90$ °C) and under poor solvent conditions ($\square$, $T = 31.82$ °C) after a 3-day standing time, where $\theta = 15°$.
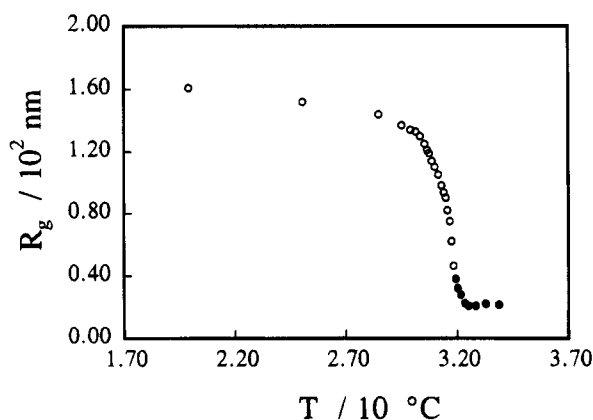
Figure 2. Plot of the radius of gyration R_g vs temperature for the same solution used in Figure 1, where all values of R_g were determined in the scattering angle range of 6—22° with a step increment of 0.5°.

Figure 1 shows two typical hydrodynamic radius distributions $f(R_h)$ of the dilute PNIPAM aqueous solution under good solvent conditions (O, $T = 19.90$ °C) and under poor solvent conditions ($\square$, $T = 31.82$ °C) after standing for ~3 days, where $\theta = 15°$. Further, our dynamic LLS results showed that at $T = 31.82$ °C the hydrodynamic radius distribution was independent of time and there was no aggregation observed even after a long standing time, which clearly implies that the PNIPAM solution at $T = 31.82$ °C is a thermodynamically stable system. The average hydrodynamic radii $\langle R_h \rangle$ of these two $f(R_h)$ s are respectively 54.1 and 112 nm. It shows a clear collapse of the PNIPAM chain in water. On average, the hydrodynamic size of the PNIPAM chain has shrunk about twice which corresponds to a ~10-fold hydrodynamic volume change. A more pronounced size or volume change of the PNIPAM chain has been observed in terms of the radius of gyration R_g measured in static LLS.

Figure 2 shows the change of R_g as a function of temperature for the extremely dilute PNIPAM solution, where all values of R_g were determined in the scattering angle range of 6—22° with a step increment of 0.5°. This small accessible scattering angle range had ensured the condition of $R_g q < 1$. The filled circles in Figure 2 represent the data obtained in a thermodynamically instable state, i.e., in a kinetic chain collapse process. A similar study has been carried out for polystyrene in cyclohexane.[14] This kinetic collapse process has been


Figure 3. Typical hydrodynamic radius distribution of the same PNIPAM solution used in Figure 1 after the solution was brought from 30.59 °C (Θ-temperature) to 33.02 °C and stood at 33.02 °C for 4 h, where $\theta = 15°$.

theoretically predicted to involve the following four possible processes: the crumpling, knotting, aggregation, and interpenetration of polymer chains.[13] The time interval between the chain crumpling (and knotting) and the aggregation of the collapsed chains was mainly dependent on the temperature difference between the solution temperature and the Flory Θ-temperature, i.e., $T - \Theta$. A typical time interval for PNIPAM in water at 32 °C < T < 34 °C is in the range of ~10^2–10^3 min, which is very similar to that observed in the polystyrene/cyclohexane system.[14]

Figure 3 shows a typical hydrodynamic radius distribution of the PNIPAM chains in water after the solution was brought from 30.59 °C (the Θ-temperature) to 33.02 °C and stood at 33.02 °C for ~4 h. In comparison with Figure 1 where the hydrodynamic radius distribution at $T = 31.82$ °C has only one narrow distribution peak, Figure 3 shows a bimodal distribution with two broadly distributed peaks. The peak with a smaller size mainly represents individual collapsed chains, and the peak with a higher size indicates that there exists a very small amount of larger aggregates made of the collapsed chains in the solution. Figure 3 also reveals that at $T = 33.02$ °C the PNIPAM chains were in a more collapsed state in comparison with that at $T = 31.82$ °C. The peak broadening after a ~4-h standing time might be due to the two following processes: (1) the "knotting" (or more generally "rearranging") of the collapsed chain which leads to a more compact and smaller size; (2) the formation of some small aggregates, such as dimer, trimer, and so on, which leads to a slightly larger size. Further aggregation of these small aggregates leads to a second large aggregate peak in Figure 3. This is why in Figure 2 we have filled those experimental data points obtained at $T > 31.82$ °C since dynamic LLS results showed that the PNIPAM chain is not thermodynamically stable in water in that temperature range.

Figure 4 shows a master curve of the scaled expansion factor of the static size (i.e., R_g) "$\alpha_s{}^3 M_w{}^{1/2}\tau$ vs $M_w{}^{1/2}\tau$", where $\alpha_s = R_g(T)/R_g(\Theta)$ and $\tau = (T - \Theta)/\Theta$. Hollow circles represent the results obtained in thermodynamically stable states, and filled circles, the results obtained just after the temperature reached equilibrium where the PNIPAM chains already collapsed but the aggregation between these collapsed chains had not started yet. The general shape of this master curve has been predicted in theory. The ratios of R_g/R_h at different temperatures are also indicated in Figure 4 on the
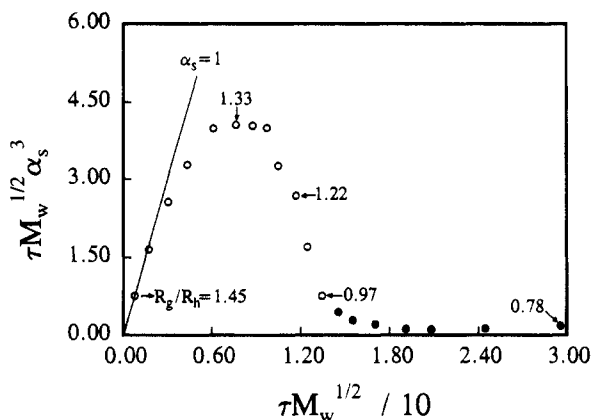
Figure 4. Master curve of the scaled expansion factor of the static size (the radius of gyration, R_g) $\alpha_s^3 M_w^{1/2}\tau$ vs $M_w^{1/2}\tau$, where $\alpha_s = R_g(T)/R_g(\Theta)$ and $\tau = (T - \Theta)/\Theta$, with Θ being the Flory Θ-temperature ($\Theta = 30.59$ °C). Hollow circles represent the experimental results obtained in thermodynamic stable states ($T \leq 31.82$ °C); and filled circles, the results obtained just after the temperature reached equilibrium where the PNIPAM chains already collapsed but the aggregation between these collapsed chains had not started yet.

master curve. It is well-known that for a linear polymer coil in a good solvent and a uniform hard sphere the R_g/R_h values are ~1.5 and ~0.77, respectively.[24] The change of the R_g/R_h ratio from 1.45 to 0.77 clearly shows that the PNIPAM chain in water had collapsed from a typical expanded flexible polymer coil into a compact globule with a uniform density when temperature increased from 20.0 to 34.0 °C. On the basis of the clear deviation of the master curve away from the plot of $\alpha_s = 1$ and the listed R_g/R_h ratio, we suggest that the master curve be divided into the four following stages: (1) the chain contracting (the initial raising part) where R_g/R_h changes from 1.45 to 1.33; (2) the chain crumpling (the plateau part) where $R_g/R_h \sim 1.33$; (3) the chain knotting or rearranging (the falling part) where R_g/R_h decreases sharply from 1.33 to 0.97; and (4) the chain collapsing into a compact globule with a uniform density (the last level-off part) where R_g/R_h approaches a constant value. At $T = 31.82$ °C, which corresponds to the last observed thermodynamically stable state for the PNIPAM chain in water, $R_g/R_h = 0.97$ which indicates that the PNIPAM chain has reached a globule state, even though the PNIPAM chain has not become a compact globule with a uniform density. Moreover, the extended plateau (the crumpled globule) and the following decay (the thermodynamically stable knotted globule) in the master curve $\alpha_s^3 M_w^{1/2}\tau$ vs $M_w^{1/2}\tau$ has been observed for the first time, to our knowledge, which has to be attributed to several unique features of the PNIPAM sample used in this study, namely, ultrahigh molecular weight, very narrowly distributed molecular weight, and extremely dilute concentration. In the past, such an extended plateau was only observed in the

master curve of the scaled expansion factor of the dynamic size (R_h) $\alpha_h^3 M_w^{1/2}\tau$ vs $M_w^{1/2}\tau$ for PS in organic solvents, where $\alpha_h = R_h(T)/R_h(\Theta)$.

In conclusion, a thermodynamically stable globule state of a single polymer chain has been experimentally realized with an extremely dilute solution (~5 µg/mL) of a narrowly distributed high molecular weight PNIPAM sample ($M_w = 1.08 \times 10^7$ and $M_z/M_w < 1.06$) in water. Our experimental results support a four-stage coil-to-globule transition process for a single PNIPAM chain in water, namely, the chain contraction, crumpling, knotting, and collapsing. The study of an even higher molecular weight PNIPAM is underway. The collapsing kinetics and comparison between the coil-to-globule transition of individual free PNIPAM chains in water and the lightly cross-linked PNIPAM chains in microgel particles will be reported in an upcoming paper.

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