How Fast a Long Linear Flexible Homopolymer Chain Collapses When Solvent Switches from Good to Poor

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ABSTRACT: The kinetics of the coil-to-globule transition of individual long linear flexible poly(*N*-isopropylacrylamide) (PNIPAM) homopolymer chains (1.5×10^7 g/mol) in different water/methanol mixtures with a polymer concentration as low as 5×10^{-7} g/mL was studied by the stopped-flow method. To avoid possible interference of labeling the chains with fluorescence probes, we used pyrene free in water as a probe to monitor the change of the chain conformation. Our results reveal, for the first time to our knowledge, that the characteristic transition time (τ_c) of such long homopolymer PNIPAM chains is on the order of ~10 ms and increases with the quenching depth and chain length.

Introduction

The coil-to-globule transition is a fundamental problem in polymer physics because it is related to many phenomena, such as protein folding and DNA packing. It was predicted long time ago that the conformation of a long flexible homopolymer chain could change from a random coil to a compact globule when the solvent quality changes from good to poor.[1−6] Most of the past studies focused on whether such a transition is feasible. In the past 10 years, we have confirmed the existence of such a coil-to-globule transition.[7−10] But how fast a long homopolymer chain collapses in a poor solvent is still a remaining problem. Few kinetic studies in the past only showed that such a chain-folding process is faster than the change of solvent quality in laser light scattering, such as the temperature jump. Chu et al.[11] showed that the collapse of long polystyrene chains followed a two-stage kinetics with two characteristic relaxation times on the order of $\sim 10^2$ s.

Theoretically, de Gennes[12] suggested that if a polymer chain were quenched in a poor solvent, the chain would adopt a string "sausage-like" conformation, and each "sausage" would gradually shorten and thicken in a self-similar manner during the collapsing process. With a normal solvent viscosity, the estimated characteristic folding time (τ_c) would be on the order of 10 ms, much faster than the observed value of $\sim 10^2$ s. Such a discrepancy was attributed to a higher local viscosity in the volume occupied by the collapsed polymer chain and/or to some possible topological constraints, such as the self-entanglement in the collapse state.[11] Recently, we revisited such an existing problem by using a stopped-flow device with a fluorescence detector to investigate the kinetics of the coil-to-globule transition of individual linear polymer chains in dilute solutions.

Note that the stopped-flow technique has been widely used to study the kinetics of the micellar formation[13] and the protein folding.[14] It normally has a time resolution of a few milliseconds. To slow down the kinetics of the coil-to-globule transition to the measurable range, we have to use long linear poly(*N*-isopropylacrylamide) (PNIPAM) homopolymer chains. It has been known that both pure methanol and pure water are good solvents for PNIPAM at room temperature, but a certain mixture of methanol and water (~15−50% v/v) is a poor solvent for PNIPAM.[15−17] They are different from either pure water or pure methanol in nature due to their complexation. Therefore, mixing a proper amount of water with its methanol solution can induce the coil-to-globule transition of linear PNIPAM chains. In this study, to avoid possible interference of labeling a polymer chain with a hydrophobic fluorescence probe as done in a previous study,[18] a trace amount of free pyrene as a probe was added in the solution because its fluorescent intensity is very sensitive to the hydrophobicity and polarity of its surrounding, reflecting the intensity ratio of the first and the third emission maximum (I_1/I_3).[19] Namely, the I_1/I_3 ratio decreases when its surrounding changes from hydrophilic to hydrophobic.[20,21] We have tested that the addition of a small amount of pyrene has nearly no effect on the chain conformation. Varying the methanol/water ratio, we can alter the quenching depth.

Experimental Section

Sample Preparation. The synthesis of long PNIPAM chains was detailed before.[7] The resultant PNIPAM homopolymer was carefully fractionated by three cycles of the dissolution/precipitation process in a mixture of dry acetone and dry hexane at the room temperature. Static laser light scattering (LLS) measurements showed that the weight-average molar mass (M_w) of the PNIPAM fraction used here was $(1.5 \pm 0.1) \times 10^7$ g/mol. The polydispersity index ($M_w/M_n \sim 1.2$) was estimated from the relative width $\mu_2/\langle\Gamma\rangle^2$ of the line-width distribution measured in dynamic LLS by using $M_w/M_n \sim 1 + 4\mu_2/\langle\Gamma\rangle^2$.[22]

Pyrene was first dissolved in acetone with a concentration of $\sim 10^{-5}$ g/mL. After acetone was removed by vacuum at the room temperature, 20 mL of methanol solution of PNIPAM (1.0×10^{-5} g/mL) was added into the bottle. In such a way, pyrene became soluble in methanol, and its final concentration was 5.0×10^{-7} g/mL. Deionized water was used as another solvent. Both the PNIPAM/pyrene solution and water were clarified by a 0.45 μm

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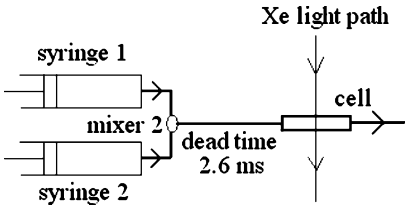

Figure 1. Schematic of the stopped-flow device used and its liquid paths inside.

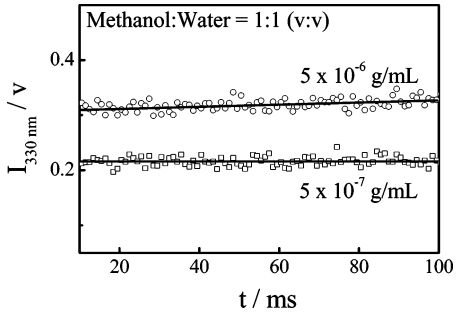

Figure 2. Time dependence of intensity of light (I) scattered at a right angle after mixing the solution of PNIPAM in methanol with deionized water for two different polymer concentrations.

Millipore Millex-LCR filter to remove dust prior to the stopped-flow experiment.

Stopped-Flow Measurement. The rapid kinetic instrumentation is equipped with a MOS-250 fast spectrometer, a SFM-300/S stopped-flow mixer, a 150 W (Xe) lamp as the light source, and a fast photomultiplier detector. Figure 1 schematically shows such a device and its liquid paths. Two stepping motors were used to pump syringes 1 and 2, respectively. The excitation wavelength was 333 nm. The emission spectra were measured in the range 350−450 nm. Both the emission and excitation slit widths were 5 nm. In the static measurement, the scanning rate was 60 nm/min. The flowing time between the mixer 2 and the middle of the observation cell is the instrument dead time (2.6 ms). The syringes and the cell were kept at 25.0 ± 0.1 °C. The methanol solution of PNIPAM/pyrene and deionized water were respectively stored in syringes 1 and 2. The volume ratio of the methanol solution and water was controlled by different flowing rates of syringes 1 and 2, but the total flowing rate was kept at a constant (14 mL/s). After each shot, the fluorescence intensities of the two peaks (I_1 and I_3) of pyrene in the solution mixture were monitored. The delay time between the shot and the detection was 10 ms. An electronics valve designed for the rapid kinetic experiment was used to vent the solution mixture after each measurement.

Results and Discussion

We first examined the concentration at which individual PNIPAM chains can undergo the coil-to-globule transition to form single-chain globules without interchain association. To do so, we set both the excitation and detection wavelengths at 330 nm to measure the scattering intensity at right angle. Figure 2 shows the time dependence of the scattering intensity (I) after the PNIPAM/methanol solution was mixed with water. Note that the scattering intensity is very sensitive to the chain association because it is proportional to the mass (M) of a scattering object for a given concentration. Figure 2 shows that the scattering intensity after the mixing slightly increases when the PNIPAM concentration was $\sim 5 \times 10^{-6}$ g/mL but nearly remained a constant when the solution was further diluted 10 times. We can estimate the average collision time (τ_{collis}) between two polymer chains in such a dilute solution by[23]

$$\tau_{collis} \sim l^2/D \quad (1)$$

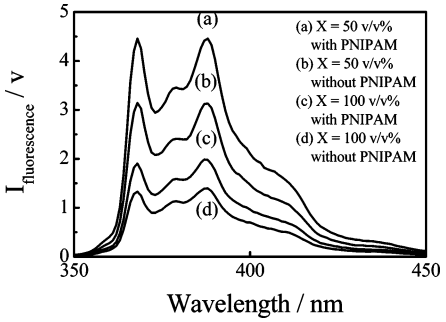

Figure 3. Static emission fluorescence spectra of pyrene (5×10^{-7} g/mL) in different solutions or solution mixtures with different methanol contents (X).

where l is the average distance between two chains, related to the polymer concentration (C) as $l \sim C^{-1/3}$, and D is the average traditional diffusion coefficient of polymer chains in the solution. There are $\sim 2 \times 10^9$ chains per cm^3 in such a dilute solution. D measured in dynamic light scattering is 6.78×10^{-8} cm^2/s. Therefore, $\tau_{collis} \sim 2$ s, much longer than the observation time window ($\sim$100 ms) used in this study. It should also be noted that the total time required for the mixing and flowing from the mixing chamber to the detection cell is no more than 10 ms. The polymer chains in such a dilute solution should have no time or much less chance to undergo the interchain association in this process. In other words, we can ignore interchain association within the first 100 ms after the mixing when such a dilute solution was used to study the coil-to-globule transition of individual chains.

Figure 3 shows the methanol content (X) dependence of the emission fluorescence spectra of pyrene ($C = 5.0 \times 10^{-7}$ g/mL) in different solutions with and without PNIPAM in static measurements. For pyrene in pure methanol, in the mixture of methanol and water (1:1, v/v) and in pure water, the ratios of I_1/I_3 are 1.16, 1.31, and 1.50, respectively. Both I_1 and I_3 increase with the water content, but the increase of I_1 is faster than I_3 because water is more polar than methanol. For $X = 100\%$ and 50% v/v, I_1/I_3 in the presence of PNIPAM (5.0×10^{-7} g/mL) was 1.16 and 1.27, respectively. It is clear that in pure methanol the presence of a small amount of PNIPAM has no effect on I_1/I_3. Note that PNIPAM is soluble in pure methanol and exists as individual swollen coiled chains. Also note that PNIPAM is more hydrophobic than methanol because of its chain backbone. Therefore, pyrene should be near or around PNIPAM, but not binding to it. When $X = 50\%$ v/v, the slightly lower value of I_1/I_3 in the presence of PNIPAM can be attributed to the partition of some pyrene molecules inside the collapsed PNIPAM chains because they are dehydrated and more hydrophobic and less polar than water.[17]

Figure 4 shows the relative change of the fluorescent intensity ratio $(I_1/I_3)_{relative}$, defined as $[(I_1/I_3)_t - (I_1/I_3)_\infty]/[(I_1/I_3)_0 - (I_1/I_3)_\infty]$, under two different situations, where the subscripts "t", "∞", and "0" denote different times: $t = t$, $t \rightarrow \infty$, and $t = 0$, respectively. Without PNIPAM, the mixing of pure methanol and pure water leads to an expected quick and sharp increase of $(I_1/I_3)_{relative}$ with $\tau_c > 1$ ms because water is more polar than methanol. Such a fast process can be attributed to the quick interdiffusion of methanol and water. In the presence of PNIPAM, the mixing of the methanol solution and water results in a gradual decrease of $(I_1/I_3)_{relative}$ with $\tau_c \sim 17$ ms, slower than the methanol/water interdiffusion. The decrease of $(I_1/I_3)_{relative}$ shows that some of the pyrene molecules must be entrapped inside individual collapsed PNIPAM chains.

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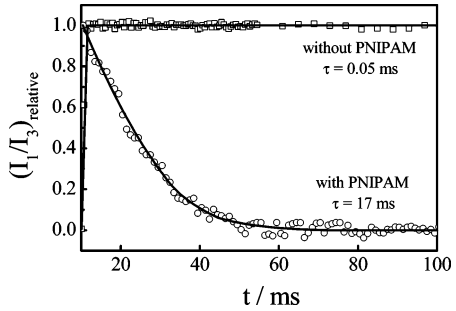

Figure 4. Time dependence of relative change of fluorescent intensity ratio $(I_1/I_3)_{relative}$ under different situations, where $(I_1/I_3)_{relative}$ is defined as $[(I_1/I_3)_t - (I_1/I_3)_\infty]/[(I_1/I_3)_0 - (I_1/I_3)_\infty]$ where the subscripts "t", "∞", and "0" respectively denote different times $t = t$, $t \to \infty$, and $t = 0$ after the mixing, The symbols (□) are for mixing water with a solution of pyrene in methanol without PNIPAM, and (○) are for mixing water with a solution of pyrene in methanol with PNIPAM.

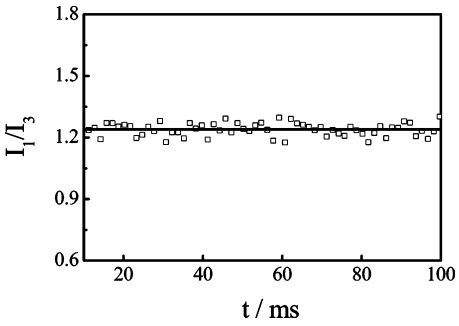

Figure 5. Time dependence of fluorescent intensity ratio (I_1/I_3) after mixing a solution of PNIPAM in water/methanol (v:v = 1:1, without pyrene) with a solution of pyrene in water/methanol (v:v = 1:1, without PNIPAM).

The question is whether these pyrene molecules were originally near and around the hydrophobic PNIPAM chain backbone so that they were entrapped inside during the chain folding or they diffused into these folded chains afterward; i.e., the collapse of individual PNIPAM chains was much fasten than the possible diffusion. To differentiate these two possibilities, we mixed one water/methanol solution (v:v = 1:1) with pyrene but without PNIPAM and another water/methanol (v:v = 1:1) solution without pyrene but with the collapsed PNIPAM chains. Figure 5 shows that in this case $(I_1/I_3)_t$ remains as a constant; i.e., pyrene cannot diffuse into the collapsed PNIPAM chains within the observation time window (∼100 ms). In other words, the decrease of I_1/I_3 in Figure 4 reflects that pyrene molecules must be near or around the PNIPAM chains so that they can be entrapped during the coil-to-globule transition. Note that for a higher PNIPAM concentration (5.0×10^{-6} g/mL) the mixing also led to a similar decrease (not shown).

The change of $(I_1/I_3)_{relative}$ after the mixing can be rationally summarized as follows. In the methanol solution of PNIPAM, pyrene molecules are near or around the PNIPAM chains that are more hydrophobic and less polar in comparison with methanol. The mixing leads to a sudden change of the environmental polarity, resulting in a quick increase of $(I_1/I_3)_{relative}$, and at the same time, a relatively slower collapse of individual PNIPAM chains starts because the methanol/water mixture is a nonsolvent for PNIPAM. Pyrene molecules near or around the PNIPAM chains are entrapped during the collapsing process. The collapsed chains provide a more hydrophobic and less polar environment for pyrene so that $(I_1/I_3)_{relative}$ decreases after the initial jump.

Figure 4 shows that it takes ∼60 ms to complete such a chain-folding process in the solution studied. de Gennes[12] predicted

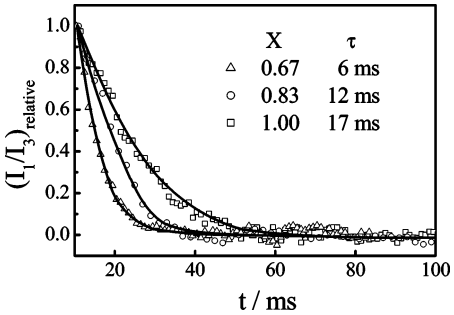

Figure 6. Methanol content (X) dependence of relative change of fluorescent intensity ratio $(I_1/I_3)_{relative}$ after mixing deionized water with a solution of PNIPAM in methanol (5×10^{-7} g/mL), where $(I_1/I_3)_{relative}$ is defined in Figure 4 and the text.

that for homopolymer chains the characteristic time (τ_c) of the coil-to-globule transition is related to the Boltzmann constant (k_B), the solvent viscosity (η), the Θ-temperature, the size of a link (a), the total number of links (N), and the quench depth ΔT ($\equiv |\Theta - T|$) as

$$\tau_c \sim \frac{\eta a^3}{k_B \Theta} N^2 \frac{\Delta T}{\Theta} \qquad (2)$$

For $a \sim 0.5$ nm, $N \sim 10^5$, $\eta \sim 10^{-2}$ P, and $\Delta T/\Theta \sim 0.1$, $\tau_c \sim$ 10 ms, fairly close to the measured characteristic decay time in Figure 4. Our preliminary results on different chain lengths showed that τ_c is also proportional to N^2 as predicted. However, only one relaxation was observed. Our previous static studies showed that the collapsed homopolymer chains are in the crumpled state, and the local concentration was as high as 30−40%.[7−10] It is difficult for such a long chain in such a high concentration to relax. Therefore, the predicted second stage might be well beyond our time window. As we previously stated,[17,18] the two-stage kinetics observed for the hydrophobically modified copolymer chains might be attributed to the transitions, namely, from a random coil to an ordered coil and from the ordered coil to the final folded core−shell structure.

Note that τ_c also increases with the quenching depth (ΔT). In the present study, instead of changing the solution temperature, we varied the methanol content to reach different quenching depths. Most likely, the solution was in the metastable state. Previously, we showed that the ratio of the average radius of gyration to the average hydrodynamic radius (R_g/R_h) of individual PNIPAM chains decreases from 1.05 to 0.75 when the volume ratio of methanol to water increases from 0.67 to 1.00 because of different degrees of the chain shrinking.[15,16] To test this point, we varied the ratio of methanol and water but kept the final PNIPAM concentration in the solution mixture to be a constant (5×10^{-7} g/mL). Figure 6 shows that the characteristic decay time (τ_c) indeed increases with the methanol content.

Conclusion

The stopped-flow technique with fluorescence detection can be effectively used to study the kinetics of the coil-to-globule transition of individual long homopolymer chains in dilute solutions. Our results shows that in an extremely dilute solution (5×10^{-7} g/mL) individual poly(*N*-isopropylacrylamide) (PNIPAM) chains ($M_w \sim 1.5 \times 10^{-7}$ g/mol) can undergo the coil-to-globule transition to form individuated single-chain globules without any noticeable interchain association within the observation time window (∼100 ms). The characteristic folding time of such long PNIPAM chains in the methanol/

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water (1:1 v/v) mixture is ~10 ms. As expected, the shrinking time required becomes shorter when the quenching depth decreases.

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