First Observation of the Molten Globule State of a Single Homopolymer Chain

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In a recent laser light-scattering study on the "globule-to-coil" transition (melting) of a single poly(*N*-isopropylacrylamide) (PNIPAM) linear chain in water (the solution is in the stable one-phase region at all temperatures), we observed that at the initial melting stage the ratio of the hydrodynamic radius to the radius of gyration underwent an unexpected maximum ($\sim$1.61) which is even higher than $(5/3)^{1/2}$ predicted for a rigid and uniform globule, indicating a molten globule. This finding suggests that just as with a protein molecule the melting of a homopolymer globule is not an all-or-none process and the molten state is a general phenomenon for macromolecules in solution. [S0031-9007(96)01345-2]

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In recent years, there has been a considerable interest in the study of the molten globule state of proteins [1–6]. It has been assumed that a protein molecule at the molten globule state has a secondary structure, but with no rigid tertiary structure [1]. It has also been suggested that this molten globule is not an unfolded chain with a local secondary structure, but a distinct physical state of a protein molecule with a special combination of properties of native and unfolded proteins [1]. This molten globule state is believed to involve a number of physiological processes in the living cell [7]. One of the expected properties of this molten globule is a dense "core" and a molten "shell," or, in other words, its density decreases from the center of the globule to the periphery. However, a direct study of this molten globule state without large admixtures of either native or unfolded states is very difficult.

On the other hand, as a fundamental problem in polymer physics, the "coil-to-globule" transition of a linear synthetic homopolymer chain not only resembles the folding of a protein molecule [8], but also provides a more defined model system for the study of the protein folding. However, the experimental success in this research area is very limited due to two extreme requirements of the polymer chain; namely, its molar mass should be higher than 10^7 g/mol and its polydispersity index (M_w/M_n) should be less than 1.1, where M_w and M_n are the weight- and number-averaged molecular weights, respectively. Only in a recent study, we have, for the first time, realized the thermodynamically stable globule state of a single poly(*N*-isopropylacrylamide) (PNIPAM) linear chain in water [9]. As a homopolymer, PNIPAM is a chemical isomer of poly(leucine), but with a polar peptide group in the side chain rather than in the backbone. On the basis of the study of the coil-to-globule transition, we further studied the melting (the "globule-to-coil") of a PNIPAM globule using laser light scattering and observed the molten globule state of a single PNIPAM chain. The experimental details are as follows.

An extremely dilute PNIPAM ($M_w = 1.21 \times 10^7$ g/mol and $M_w/M_n < 1.05$) aqueous solution ($C \sim 10^{-6}$ g/mL) was prepared by a combination of fractionation and filtration [9]. The successful preparation of such a PNIPAM solution is an essential step in the study of the coil-to-globule transition. A modified commercial laser light scattering (LLS) spectrometer (ALV/SP-125) equipped with a multi-τ digital correlator (ALV-5000) and a solid-state laser (ADLAS DPY425II, output power $\sim$400 mW at $\lambda_o =$ 532 nm) was used. The modified LLS spectrometer has an unusual small angular range of 6°–15° which is particularly useful in studying a longer polymer chain [9].

In static LLS, the root-mean square *z*-average radius ($\langle R_g^2 \rangle_z^{1/2}$ or written as $\langle R_g \rangle$) of the polymer chain in solution can be determined from the angular dependence of the access absolute time-averaged scattered light intensity [i.e., the Rayleigh ratio, $R_{vv}(q)$] on the basis of $KC/R_{vv}(q) \approx (1/M_w)[1 + (1/3)\langle R_g^2 \rangle q^2]$, where $K = 4\pi^2 n^2 (dn/dC)^2/(N_A \lambda_o^4)$ and $q = (4\pi n/\lambda_o)\sin(\theta/2)$ with n, dn/dc, N_A, λ_o, and θ being the solvent refractive index, the specific refractive index increment, Avogadro's number, the wavelength of light in vacuum, and the scattering angle, respectively [10].

Figure 1 shows a typical angular dependence of $KC/R_{vv}(q)$ for individual PNIPAM chains in water at 31.42 °C, where the line represents a least-square fitting. $\langle R_g \rangle$ can be calculated from the ratio of the slope to the intercept of the line. The inset shows the temperature dependence of $\langle R_g \rangle_T/\langle R_g \rangle_o$ in the globule melting process, where $\langle R_g \rangle_T$ and $\langle R_g \rangle_o$ are the average radiuses of gyration of individual PNIPAM chains at the temperature T and in the globule state, respectively. It should be stated that PNIPAM in water has a low critical solution temperature (LCST); i.e., at a temperature lower than 30.58 °C water is a good solvent for PNIPAM so that the PNIPAM chain has a random-coil conformation, whereas at a higher temperature the PNIPAM chain collapses into a globule. It can be seen that $\langle R_g \rangle$ increases $\sim$8 times when the PNIPAM chain changes from a globule state to an unfolded random-coil state, implying a change of $\sim$500 times in the accessible space of the PNIPAM chain.

In dynamic LLS, for a diffusive relaxation, the linewidth distribution, $G(\Gamma)$, calculated from the Laplace inversion of the measured intensity-intensity time correlation

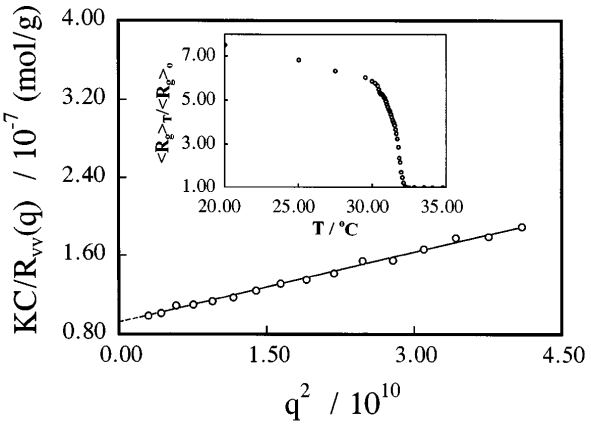

FIG. 1. Typical angular dependence of $KC/R_{vv}(q)$ for individual PNIPAM chains in water at 31.42 °C. The inset shows the temperature dependence of $\langle R_g\rangle_T/\langle R_g\rangle_o$ in the globule melting process, where $\langle R_g\rangle_T$ and $\langle R_g\rangle_o$ are the average radiuses of gyration of individual PNIPAM chains at the temperature T and in the globule state, respectively.

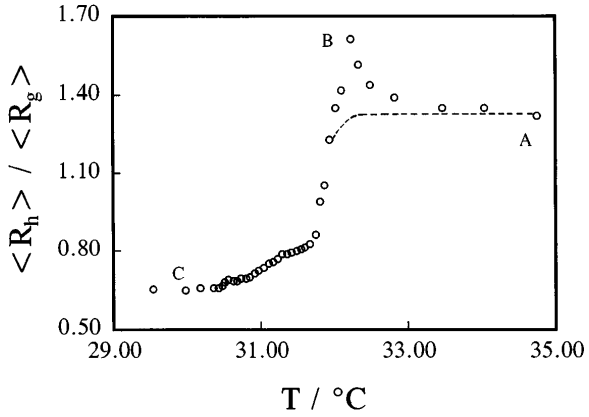

FIG. 3. The temperature dependence of $\langle R_h\rangle/\langle R_g\rangle$ of individual PNIPAM chains in water in the globule melting process, where $\langle R_h\rangle$ and $\langle R_g\rangle$ are the hydrodynamic radius and the radius of gyration, respectively. *A*, *B*, and *C* indicate three different physical states schematically represented in Fig. 4.

function can lead to a hydrodynamic radius distribution, $f(R_h)$, and further an average hydrodynamic radius, $\langle R_h\rangle$ $[= \int_0^\infty f(R_h)R_h\,dR_h]$ [11–13]. Figure 2 shows the temperature dependence of $\langle R_h\rangle_T/\langle R_h\rangle_o$, where $\langle R_h\rangle_T$ and $\langle R_h\rangle_o$ are the average hydrodynamic radius of individual PNIPAM chains at the temperature T and in the globule state, respectively. In comparison, $\langle R_h\rangle$ only increases ~4 times in the globule melting process. The ratio of $\langle R_h\rangle/\langle R_g\rangle$ reflects a polymer chain conformation.

Figure 3 shows the temperature dependence of $\langle R_h\rangle/\langle R_g\rangle$ in the globule melting process. The change of $\langle R_h\rangle/\langle R_g\rangle$ from ~1.30 to ~0.66 agrees well with the values predicted for a rigid uniform sphere (~1.29) and a random-coil polymer chain in good solvent (~0.66). If the melting was an all-or-none process, the change of $\langle R_h\rangle/\langle R_g\rangle$ in Fig. 3 would follow the dashed line. However, it showed an unexpected maximum value of ~1.61 at $T =$ 32.23 °C, suggesting the existence of another physical state between the fully collapsed globule and the unfolded random coil. This is identified as the molten globule state of a single PNIPAM chain in water, resembling the molten globule state of a protein molecule. It should be noted that the solution is in the stable one-phase region at all temperatures.

Figure 4 shows a schematic of three different states (*A*, *B*, and *C*) of a single PNIPAM chain in the globule melting process. *A* and *C* (also indicated in Fig. 3) represent the initial globule and the final random-coil states of a single PNIPAM chain, respectively. Both *A* and *C* have a uniform chain density, but the density of *A* is much higher. It is worth noting that in *C* there is a hydrodynamic draining so that its $\langle R_h\rangle/\langle R_g\rangle$ is much smaller. In the middle of $A \rightarrow C$, there is the molten globule state *B*. Larger $\langle R_h\rangle/\langle R_g\rangle$ value indicates that $\langle R_h\rangle$ increases faster than

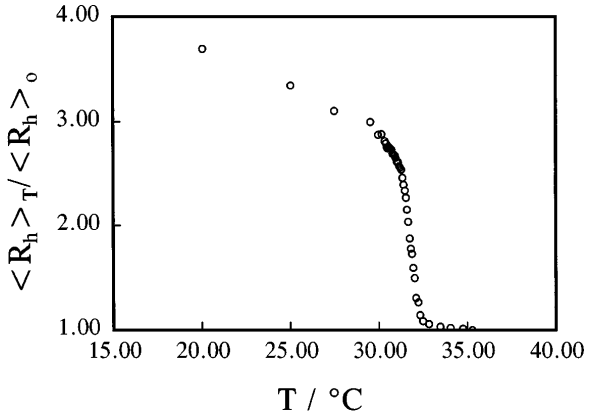

FIG. 2. The temperature dependence of $\langle R_h\rangle_T/\langle R_g\rangle_o$ in the globule melting process, where $\langle R_h\rangle_T$ and $\langle R_g\rangle^*$ are the average hydrodynamic radius of individual PNIPAM chains at the temperature T and in the globule state, respectively.

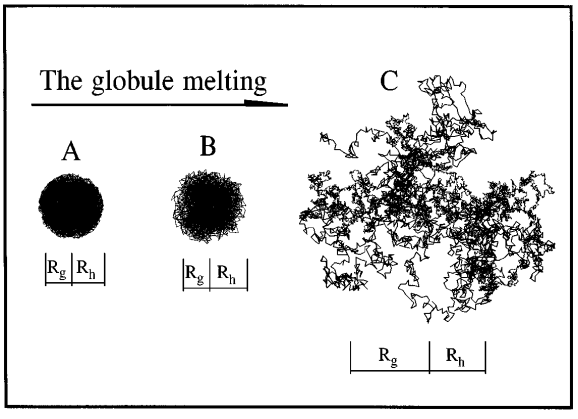

FIG. 4. Schematic of three different physical states of a single PNIPAM chain in the globule melting process. *A*: an initial globule before the melting; *B*: a molten globule; and *C*: an unfolded random coil.

$\langle R_g \rangle$ and the melting is not uniform; namely, the portion near the periphery of the globule (the shell) melts faster than the inner portion of the globule (the core). In this way, the "core" has a chain density similar to that in *A*, but the "shell" has a lower chain density. Since the degree of the melting at this stage is small, it is expected that $\langle R_g \rangle$ is nearly a constant and only $\langle R_h \rangle$ increases when the globule melts. Therefore, $\langle R_h \rangle / \langle R_g \rangle$ increases at this stage instead of following the dashed line shown in Fig. 3. This nonuniform chain density resembles the molten globule state of a protein molecule and indicates that the melting of a high molar mass homopolymer globule is not an all-or-none process. The observation of the molten globule state of a homopolymer globule not only supports a very important concept in the mechanism of protein folding, but also suggests that the molten globule is a physical state existing in both a larger protein molecule and a high molar mass synthetic homopolymer chain.

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