

Effect of Comonomer Distribution on the Coil-to-Globule Transition of a Single AB Copolymer Chain in Dilute Solution

ManHin Siu,[†] Guangzhao Zhang,[‡] and Chi Wu^{*,†,‡}

Department of Chemistry, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong; and The Open Laboratory of Bond Selective Chemistry, Department of Chemical Physics, University of Science and Technology of China, Hefei, Anhui, China

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ABSTRACT: Utilizing the difference between the chain conformations at two temperatures, respectively, below and above the lower critical solution temperature (~ 32 °C) of poly(*N*-isopropylacrylamide) homopolymer, we successfully prepared pairs of poly(*N*-isopropylacrylamide-*co*-vinylpyrrolidone) (NIPAM-*co*-VP) copolymers with a similar composition and chain length but different monomer distributions on the chain backbone. Using these copolymers, we investigated the temperature induced coil-to-globule transition of individual chains in dilute solution. In each case, we found that the copolymer chains could form stable single-chain globules at high temperatures, and as expected, the transition temperatures were higher than ~ 32 °C because comonomer VP is hydrophilic in the temperature range studied. The laser light-scattering and calorimetry results showed that the chains prepared at 60 °C, presumably with a globular proteinlike segmented VP distribution, had a lower transition temperature and could form denser globules than those prepared at 30 °C with a random VP distribution. In other words, the folding of the chains prepared at higher temperatures was easier. In a sense, they could “memorize” their parent globular core-shell structure with a collapsed PNIPAM core and a swollen shell made of small VP loops, reflecting in a ratio of the radius of gyration to hydrodynamic radius ($\langle R_g \rangle / \langle R_h \rangle$) much smaller than the 0.774 predicted for a uniform sphere.

Introduction

For a long time, protein folding has attracted much attention and still remains as a mystery. Protein chains contain hydrophobic, hydrophilic, and/or charged amino acid residues. The intra- and interchain hydrogen bonding and other interaction lead to some complicated bioactive structures.¹ Different theories were proposed to explain various properties of proteins from a biological point of views.^{2–5} Recently, computer simulation was also used to construct copolymers with hydrophobic and hydrophilic units to imitate proteins. Particularly, the coil-to-globule transition of different types of copolymer chains was simulated to demonstrate how the comonomer distribution, i.e., the sequence difference in structure, could greatly influence the folding of a single copolymer chain.^{6–8}

Khokhlov et al.⁸ simulated three AB copolymer chains with an identical composition and length, but different comonomer distributions on the chain backbone. Their results showed that for the chain with a globular proteinlike structure in which soluble comonomer B was incorporated on the periphery of a collapsed A chain backbone, the chain folding would be easier than that of a random copolymer without a designed sequence. Moreover, the resultant globule was stable, and its chain density was higher. The simulation suggested that such a chain could “memorize” or “inherit” some special functional properties of the parent collapsed state. Timoshenko et al.⁹ also showed that for a given degree of amphiphilicity, the folding of a AB copolymer chain with a segmented comonomer distribution was easier, and the resultant mesoglobular phase was more stable

in comparison with a random copolymer chain under the same condition.

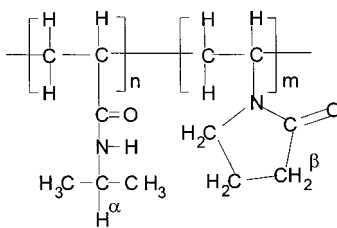
However, it is a rather difficult experimental challenge, if not impossible, to prepare a pair of AB copolymers with a similar composition and chain length, but different comonomer distributions on the chain backbone. It has been well-known that poly(*N*-isopropylacrylamide) (PNIPAM) is a thermally sensitive polymer with a lower critical solution temperature (LCST ~ 32 °C) in water.^{10–14} This interesting thermal property has made PNIPAM a simple model for the simulation of protein denaturation in aqueous solution even though real proteins are much more complicated.¹⁵ Recently, using two grafted copolymers, poly(PNIPAM-*g*-PEO), respectively, prepared at temperatures below and near the LCST of PNIPAM, Tenhu et al.¹⁶ studied the comonomer distribution dependence of the chain aggregation. Their results showed that the copolymer chains prepared at different temperatures had different lower critical solution temperatures, supporting the computer simulation in a sense. However, we should not forget that the chain aggregation is a complicate process, which involves the intrachain contraction and the interchain association. Therefore, the ultimate test and experimental challenge would be the study of the effect of comonomer distribution on the coil-to-globule transition (folding) of individual copolymer chains without involving any interchain aggregation in dilute solution.

In the last 10 years, we have accumulated much experience in the preparation of narrowly distributed long PNIPAM homopolymer chains and the study of the coil-to-globule transition of a single homopolymer chain in dilute solution.^{12–14} This enabled us to use the difference between the chain conformations at different temperatures to incorporate a second monomer into PNIPAM to obtain different sequences. In this study, hydrophilic vinylpyrrolidone (VP) was copolymerized

* The Hong Kong address should be used for correspondence.

[†] The Chinese University of Hong Kong.

[‡] University of Science and Technology of China.

Table 1. Characterization of Poly(*N*-isopropylacrylamide-*co*-vinyl pyrrolidone)s

samples	PNIPAM- <i>co</i> -VP/60/5	PNIPAM- <i>co</i> -VP/30/5	PNIPAM- <i>co</i> -VP/60/10	PNIPAM- <i>co</i> -VP/30/11
$T_{\text{synthesis}}/^{\circ}\text{C}$	60	30	60	30
area of δ_{H} H^{α}	0.874	0.875	0.775	0.745
H^{β}	0.042	0.042	0.075	0.085
[VP]/mol %	4.8	4.8	9.7	11.4
M_w (g/mol)	2.9×10^6	4.2×10^6	5.6×10^6	7.9×10^6
$T_{\text{transition}}/^{\circ}\text{C}$	33.5	35.0	36.0	37.5
$\langle R_h \rangle_{\text{collapsed}}/\text{nm}$	22.9	28.7	31.2	45.7
$\langle \rho \rangle_{\text{globule}}/(\text{g}/\text{cm}^3)$	9.6×10^{-2}	7.0×10^{-2}	7.3×10^{-2}	3.3×10^{-2}

into PNIPAM at 30 and 60 °C, which are, respectively, below and above the LCST of PNIPAM homopolymer. It was expected and a hypothesis that, at 60 °C, hydrophilic comonomer VP would segregate on the periphery of the collapsed PNIPAM segments while, at 30 °C, the copolymerization would lead to a more random distribution of VP on the PNIPAM chain. At each temperature, the copolymers with two different VP/NIPAM ratios were prepared. A proper fractionation led to long narrowly distributed copolymer chains with a similar chain length and VP/NIPAM ratio, but different comonomer distributions. The coil-to-globule transitions of these copolymer chains in dilute aqueous solution were studied by a combination of static and dynamic laser light scattering (LLS) as well as microcalorimetry.

Experimental Section

Sample Preparation. *N*-Isopropylacrylamide (NIPAM) was purified by recrystallization in a benzene/*n*-hexane mixture. 1-Vinyl-2-pyrrolidone (VP) comonomer was distilled at reduced pressure prior to use. Potassium persulfate (KPS) was purified in a mixture of water and methanol. Other chemicals were used without purification. NIPAM-*co*-VP copolymers with 5 or 10 mol % of VP were, respectively, prepared at 30 and 60 °C by free radical polymerization in water, in which KPS/*N,N,N,N*-tetramethylethylenediamine (TEMED) redox was used as initiator. Each copolymer was harvested by precipitation, i.e., pouring the reaction mixture into an equal volume of methanol. Each resultant copolymer was further purified by four cycles of redissolution in water and precipitation in methanol to ensure a complete removal of residual monomers. The final product was dried under reduced pressure at 40 °C.

The copolymer was further fractionated by precipitation from acetone solution to *n*-hexane at the room temperature. In each case, only the first fraction was used in LLS measurement. ^1H NMR (DPX 300 NMR spectrometer) was used to characterize the chain composition. The ratio of the peak areas of the methine proton of the isopropyl group in NIPAM and the two protons neighboring to the carbonyl group in VP was used to determine to the VP content. The results are summarized in Table 1, where δ_{H} is the chemical shift of proton and H^{α} and H^{β} are two protons, respectively, in the monomers of NIPAM and VP. The composition of each copolymer was close to the feeding monomer ratio prior to the copolymerization. The nomenclature used hereafter for these copolymers is NIPAM-*co*-VP/*x*/*y*, where *x* and *y* are the copolymerization temperature (°C) and the VP content (mol %), respectively. The solution with a concentration of 3.0×10^{-6} g/mL was clarified with a 0.45 μm Millipore Millex-LCR filter to remove dust before the LLS measurement. The resistivity of deionized water used was 18.0 M Ω cm.

Laser Light Scattering. A slightly modified spectrometer (ALV/DLS/SLS-5022F) equipped with a multi- τ digital time correlation (ALV5000) and a cylindrical 22 mW UNIPHASE He-Ne laser ($\lambda_0 = 632$ nm) as the light source was used. In static LLS,¹⁷ we were able to obtain the weight-average molar mass (M_w) and the *z*-average root-mean square radius of gyration ($\langle R_g^2 \rangle^{1/2}$ or written as $\langle R_g^2 \rangle$) of polymer chains in a very dilute solution from the angular dependence of the excess absolute scattering intensity, known as Rayleigh ratio $R_{\text{v}}(q)$, i.e.,

$$\frac{KC}{R_{\text{v}}(q)} \approx \frac{1}{M_w} \left(1 + \frac{1}{3} \langle R_g^2 \rangle q^2 \right) \quad (C \rightarrow 0) \quad (1)$$

where $K = 4\pi^2 n^2 (dn/dC)^2 / (N_A \lambda_0^4)$ and $q = (4\pi n / \lambda_0) \sin(\theta/2)$ with N_A , dn/dC , n , and λ_0 being the Avogadro number, the specific refractive index increment, the solvent refractive index, and the wavelength of the light in a vacuum, respectively. In this study, the solution was so dilute that the extrapolation of $C \rightarrow 0$ was not necessary. For the copolymers prepared at different temperatures and with different VP contents, dn/dC was determined by a novel differential refractometer.^{12,13} The values for PNIPAM-*co*-VP/30 and PNIPAM-*co*-VP/60 are, respectively, in the ranges 0.150–0.153 and 0.146–0.148, which weakly depend on the VP content because both homopolymers PVP and PNIPAM have a similar value of dn/dC . The variation of dn/dC in the range 25–35 °C is less than 1%, which is within the experimental error, so that we do not need to consider it in the data analysis.

In dynamic LLS,¹⁸ the Laplace inversion (the CONTIN procedure) of each measured intensity–intensity–time correlation function $G^{(2)}(q, t)$ in the self-beating mode can be related to a line-width distribution $G(\Gamma)$. For a diffusive relaxation, Γ is related to the translation diffusion coefficient D by $(\Gamma/q^2)_{C \rightarrow 0, q \rightarrow 0} \rightarrow D$, so that $G(\Gamma)$ can be converted into a transitional diffusion coefficient distribution $G(D)$ or a hydrodynamic radius distribution $f(R_h)$ via the Stokes–Einstein equation, $R_h = (k_B T / 6\pi\eta) D^{-1}$, where k_B , T , and η are the Boltzmann constant, the absolute temperature, and the solvent viscosity, respectively. The cumulant analysis of $G^{(2)}(t)$ of a narrowly distributed sample can result in an accurate average line width ($\langle \Gamma \rangle$), so that $\langle R_h \rangle$ presented in this study was determined by the cumulant analysis except stated elsewhere.

Differential Scanning Calorimeter. The copolymer solutions were measured by a VP-DSC microcalorimeter (MicroCal Inc) at an external pressure of ~ 180 kPa. The cell volume was 0.157 mL. The heating rate was 1.5 °C min^{-1} , and the instrument response time was set at 5.6 s. All the DSC data were corrected for instrument response time and analyzed using the software in the calorimeter. The polymer concentration used in DSC was kept at 1.0 mg/mL.

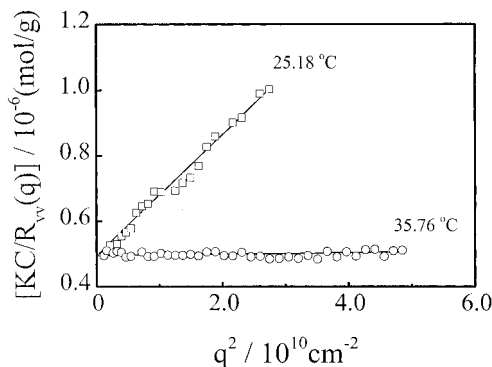


Figure 1. Scattering vector (q) dependence of Rayleigh ratio $R_v(q)$ of copolymer NIPAM-co-VP/60/5 in water, where K is a constant and the copolymer concentration (C) was 3.0×10^{-6} g/mL.

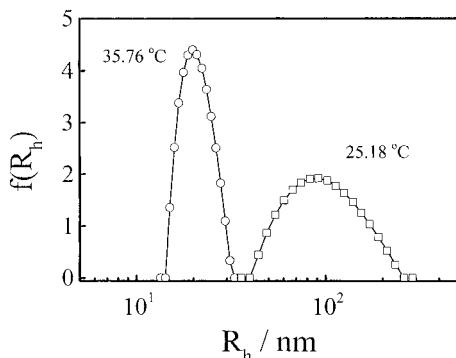


Figure 2. Temperature dependence of hydrodynamic radius distribution $f(R_h)$ of copolymer NIPAM-co-VP/60/5 in water, where the copolymer concentration was 3.0×10^{-6} g/mL.

Results and Discussion

Figure 1 shows the typical angular dependence of Rayleigh ratio $[KC/R_v(q)]$ of the copolymer chains in water at temperatures, respectively, below and above the transition temperature. On the basis of eq 1, the slope change as the temperature increases reflects the decrease of $\langle R_g \rangle$, i.e., the chain shrinking. The extrapolation of $KC/R_v(q)$ at two different temperatures led to the same intercept, clearly indicating that there was no change in the weight-average molar mass (M_w) during the shrinking process, or in other words, the process involves only individual chains. Figure 2 directly shows the shrinking of the copolymer chains at a higher temperature in terms of the shift of the hydrodynamic radius distribution of the copolymer chains in water. Apparently, the distribution width of $f(R_h)$ at ~ 25 °C is wider, but the relative widths are similar after they are normalized by the average hydrodynamic size $\langle R_h \rangle$. For each given temperature, we could obtain one $\langle R_g \rangle$ from the slope of $KC/R_v(q)$ vs q^2 and one $\langle R_h \rangle$ from hydrodynamic radius distribution $f(R_h)$ via $\int_0^\infty f(R_h) R_h dR_h$.

Figure 3 shows the temperature dependence of both $\langle R_g \rangle$ and $\langle R_h \rangle$ of two copolymers synthesized at two different temperatures with a similar chain length and identical VP content (4.8 mol %). As expected, both $\langle R_g \rangle$ and $\langle R_h \rangle$ decrease sharply during the transition, revealing the chain collapse at higher temperatures. Note that in each case, the average size of the collapsed chains remained nearly a constant even when the temperature was higher than 40 °C at which water is a very poor solvent for PNIPAM. It indicates that such formed single-chain globules were stable. In contrast, our previous study showed that for PNIPAM homopolymer

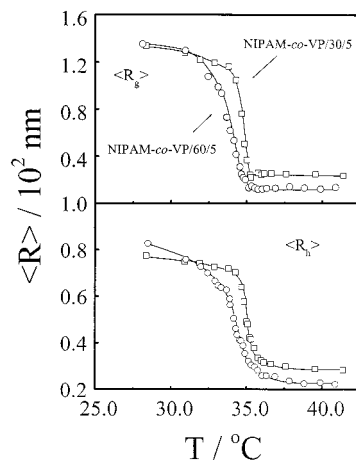


Figure 3. Temperature dependence of z -average root-mean square radius of gyration ($\langle R_g \rangle$) and average hydrodynamic radius ($\langle R_h \rangle$) of copolymers NIPAM-co-VP/60/5 and NIPAM-co-VP/30/5 in water.

chains, stable single-chain globules could only be observed within a limited temperature range.¹⁹ The formation of such stable single-chain globules can be attributed to the existence of hydrophilic comonomer VP. This is why the copolymers with hydrophilic comonomer VP have a higher transition temperature than PNIPAM homopolymer. Table 1 summarizes the coil-to-globule transition temperatures and average hydrodynamic radii in the collapsed state for two pairs of NIPAM-co-VP copolymers with different VP contents. It should be noted that the coil-to-globule transition of the copolymer chains with a higher VP content is more diffusive.

It is not surprising to see in Table 1 that the chains with a higher hydrophilic comonomer VP content have a higher transition temperature. However, it is rather interesting to see that for each pair of the copolymers with a similar VP content, the copolymer prepared at 60 °C has a lower transition temperature than its counterpart prepared at 30 °C. The difference also reflects in the average chain density of the globules; namely, in each pair, the copolymer chain prepared at 60 °C could fold into a more compact form than its counterpart. Note that $\langle \rho \rangle_{\text{globule}}$ is much smaller for PNIPAM-co-VP/30/11, which can be attributed to the higher VP content. To make sure such a shift in the transition temperature, we also measured the partial heat capacity (C_p) of these copolymers in solution using microcalorimeter. Figure 4 shows that for the two copolymers prepared at 60 °C, the temperatures at which the maximum heat capacity (T_{max}) occurs are indeed lower. Such a difference between the transition temperatures should be attributed to different comonomer distributions on the PNIPAM chain backbone because the copolymers in each pair have a similar chain length and composition.

As we mentioned before, at lower temperatures, water is a good solvent for PNIPAM and the PNIPAM segments formed during the copolymerization exist as random coil. In this way, NIPAM and VP were copolymerized into the chain in a more random fashion to form a statistical copolymer. In contrast, water at 60 °C becomes such a poor solvent that the PNIPAM segments formed must collapse and hydrophilic VP can only be incorporated into the chains on the periphery of the collapsed PNIPAM segments, leading to a segregation

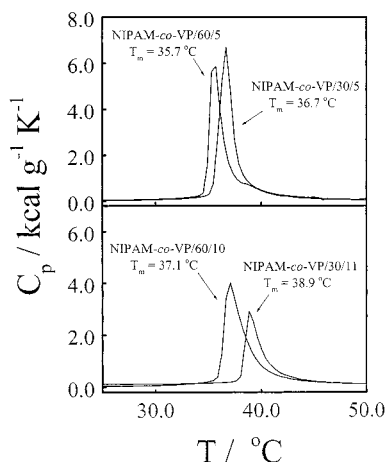


Figure 4. Temperature dependence of partial heat capacity (C_p) of NIPAM-*co*-VP copolymers in water, where the increasing rate of the temperature was 1.5 °C/min and pressure was maintained at 180 kPa.

of VP, or in other words, a segmented comonomer distribution. Therefore, the average length of the PNIPAM segment between two neighboring VP segments has to be longer in comparison with a random copolymer chain with a similar VP/NIPAM ratio, as schematically shown in Figure 5. The lower transition temperature indicates that the folding of the copolymer chains prepared at higher temperatures is easier, or in a sense, these chains could “memorize” their parent collapsed globular state.

The conformational change of a polymer chain can be better viewed in terms of the ratio of $\langle R_g \rangle / \langle R_h \rangle$ because for a coil chain and a uniform nondraining sphere, $\langle R_g \rangle / \langle R_h \rangle \sim 1.5$ and $(3/5)^{1/2}$, respectively.¹³ In Figure 6, the decrease of $\langle R_g \rangle / \langle R_h \rangle$ from ~ 1.65 to ~ 0.6 – 0.8 clear shows the coil-to-globule transition of the copolymer chains. Just like PNIPAM homopolymer in water, before fully collapsing into a uniform dense globule, the copolymer chain with a coil conformation first crumples into a molten globule characterized by a value of $\langle R_g \rangle / \langle R_h \rangle$ smaller than $(3/5)^{1/2}$ predicted for a uniform sphere, i.e.,

the dip of $\langle R_g \rangle / \langle R_h \rangle$ at ~ 35 – 36 °C. It is worth noting that even in the fully collapsed state at ~ 40 °C, the single-chain globule made of NIPAM-*co*-VP/60/5 has a ratio of $\langle R_g \rangle / \langle R_h \rangle$, much smaller than 0.774 predicted for a uniform sphere. This suggests that the globules have an uneven density distribution inside, as previously observed by Khokhlov et al.⁸ in a computer simulation.

As mentioned before, it is a hypothesis that the average length of the PNIPAM segments on a chain prepared at 60 °C is longer. Therefore, the collapse of these long PNIPAM segments could force short hydrophilic VP segments out and stay on the periphery, leading to a core-shell structure with a denser PNIPAM core and a swollen VP shell presumably made of small loops. On the other hand, the random copolymer chains prepared at 30 °C lack such segregated VP segments so that the average length of the PNIPAM segments is much shorter. The collapse of these short PNIPAM segments at high temperatures inevitably pulls the hydrophilic comonomer VP inside, resulting in a less compact, but more uniform, globule with a high ratio of $\langle R_g \rangle / \langle R_h \rangle$ similar to uniform latex particles. The comparison of the coil-to-globule transition of a pair of copolymer chains prepared at two temperatures is schematically shown in Figure 5.

The structural difference between the collapsed globules made of the chains prepared at different temperatures can also be evidenced in their size and density. Note that in Figure 3 and Table 1, when the chain is fully collapsed at ~ 40 °C, NIPAM-*co*-VP/60/5 has a smaller size than NIPAM-*co*-VP/30/5 even though they have a similar $\langle R_g \rangle$ and $\langle R_h \rangle$ in the coil state at lower temperatures. Therefore, the average densities ($\langle \rho \rangle_{\text{globule}}$) of the single-chain globules made of the chains prepared at 60 °C are higher. The values of $\langle \rho \rangle_{\text{globule}}$, defined as $M_w / (4\pi \langle R_h \rangle^3 N_A / 3)$, are listed in Table 1. The difference in $\langle \rho \rangle_{\text{globule}}$ also indirectly reflects that the copolymer chains prepared at higher temperatures can “memorize” their parent collapsed globule state and fold to a compact structure, confirming the computer simulation and prediction of Khokhlov et al.⁸ and Timoshenko et al.⁹ It should be stated that $\langle \rho \rangle_{\text{globule}}$ listed in Table 1 is

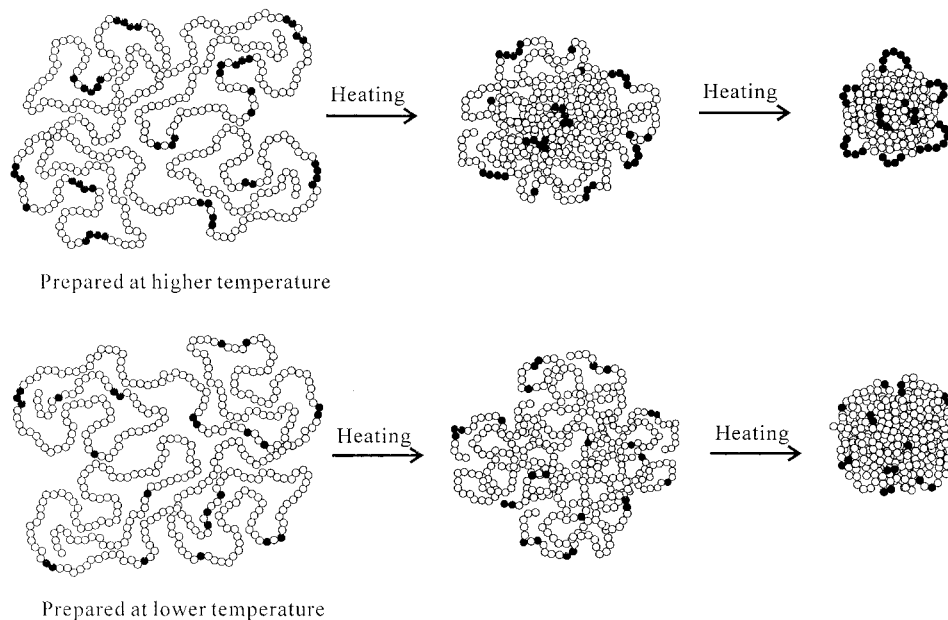


Figure 5. Schematic of different chain structures and the coil-to-globule transition of NIPAM-*co*-VP copolymers prepared at two temperatures, respectively, lower and higher than the lower critical solution temperature (LCST ~ 32 °C) of PNIPAM homopolymer.

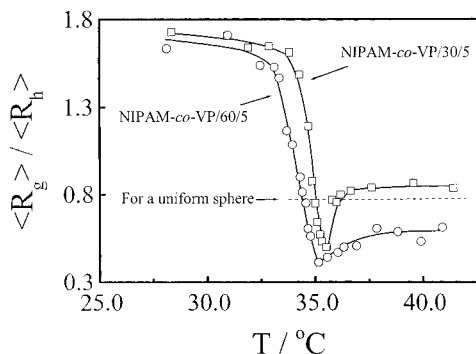


Figure 6. Temperature dependence of the ratio of average radius of gyration to the average hydrodynamic radius ($\langle R_g \rangle / \langle R_h \rangle$) of copolymer NIPAM-co-VP prepared at two different temperatures.

much lower than that of the single-chain globule made of PNIPAM homopolymer ($\sim 0.3 \text{ g/cm}^3$).¹³ This is reasonable because the incorporation of a few percent of hydrophilic comonomer VP into PNIPAM retards its close packing during the transition.

Conclusion

Using the difference between the chain conformations at different temperatures, we are able to design and prepare a pair of poly(*N*-isopropylacrylamide-co-vinylpyrrolidone) (NIPAM-co-VP) copolymers with a similar composition and chain length but different comonomer VP distributions on the chain. Especially, the copolymerization of hydrophilic VP into a PNIPAM chain at its collapsed temperature can result in a globular protein-like segmented VP distribution. The study of the temperature-induced coil-to-globule transition of these specially engineered copolymer chains have confirmed recent computer simulations; namely, the folding of a AB copolymer chain with a globular proteinlike structure is easier than the folding of a random AB copolymer chain with a similar composition and chain length. Our results also indicate that, during the coil-to-globule transition, the proteinlike chains can fold into a compact core-shell structure with a higher average chain density at high temperatures. Presumably, the core is made of collapsed long PNIPAM segments, and the shell consists of swollen short VP segments in the form of

small loops. In a sense, the copolymer coil chain formed in the collapsed state can “memorize” or “inherit” its parent globular structure when the hydrophobic interaction (attraction) between the PNIPAM segments is switched on at higher temperatures. It should be emphasized that the copolymer chains studied here are much simpler than proteins. However, this study is one step forward in a long journey toward a better understanding of protein folding.

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