The Water/Methanol Complexation Induced Reentrant Coil-to-Globule-to-Coil Transition of Individual Homopolymer Chains in Extremely Dilute Solution

Guangzhao Zhang and Chi Wu*

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

Abstract: Narrowly distributed ultralong linear poly(*N*-isopropylacrylamide) (PNIPAM) ($M_w = 2.63 \times 10^7$ g/mol and $M_w/M_n < 1.1$) was successfully prepared by a combination of fractionation and filtration. Using this PNIPAM sample, we have studied the conformation change of individual PNIPAM chains in different methanol/water mixtures at 20 °C. Both water and methanol are good solvents for PNIPAM, but not a proper mixture of them. The PNIPAM chain collapsed sharply from a coil to a stable globule when the methanol content ($x_{methanol}$) reached ~17 mol %. In the range 17−40 mol %, the PNIPAM chain remained in the globule state. Further addition of methanol finally led to a globule-to-coil transition at $x_{methanol} \sim 50$ mol %. The reentrant coil-to-globule-to-coil transition could be attributed to the formation of different water/methanol complexes, presumably, $(H_2O)_m(CH_3OH)_n$, which are poor solvents for PNIPAM. We have, *for the first time*, revealed that the conformation change of individual polymer chains can be used as an indicator to probe the complexation, similar to an indicator used in titration. Our results suggest that when $x_{methanol} < \sim17$ mol %, the water/methanol molar ratio (m/n) is ~5, while when $x_{methanol} > 50$ mol %, m/n becomes 1. In the range of ~17 mol % $< x_{methanol} < 50$ mol %, different intermediates with $1 \leq m/n \leq 5$ coexist. The speculated structures of these complexes could be related to a water pentamer.

Introduction

It has been known that poly(*N*-isopropylacrylamide) (PNIPAM) is soluble in both water and methanol at lower temperatures, but insoluble in a proper mixture of them.[1,2] Similarly, PNIPAM hydrogels can undergo a reentrant swelling−shrinking−swelling transition as the methanol content increases.[1,3−8] Utilizing this special property, one could use a mixture of two good solvents instead of one good solvent and one poor solvent to purify PNIPAM.[9,10] This special property also led to the development of some control-releasing devices for alcohol-soluble drugs.[11]

It is generally known that the reentrant transition (or cononsolvency) can be related to the demixing or the complexation of two solvents.[12] The origin of the reentrant transition of PNIPAM has long been disputed. Initially, it was attributed to the complexation between water and methanol induced by the presence of the PNIPAM network.[1,3,4] This argument was tarnished when linear PNIPAM chains in a dilute solution showed a similar reentrant phenomenon,[1,9] so that the preferential adsorption of methanol on PNIPAM was suggested because it was believed that the adsorption could induce local concentration fluctuation.[13] However, the electronic paramagnetic resonance and ^{13}C NMR studies revealed that the solvent composition at the polymer/solvent interface was conserved during the transition. Therefore, a mechanism involving both the preferential adsorption and a ternary complexation among PNIPAM, water, and methanol was proposed.[2]

On the other hand, much effort have been spent in studying the simple mixture of water and methanol, the simplest pair of molecules with hydrophobic and hydrophilic interactions. Both computer simulation[14] and experimental studies[15−21] revealed

* To whom correspondence should be addressed at The Chinese University of Hong Kong.

(1) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163−248 and the references therein.

(2) Winnik, F. M.; Ringsdorf, H.; Venzmer, J. *Macromolecules* **1990**, *23*, 2415−2416. Winnik, F. M.; Ottaviani, M. F.; Bossmann, S. H.; Garcia-Garibay, M.; Turro, N. J. *Macromolecules* **1992**, *25*, 6007−6017.

(3) Amiya, T.; Hirokawa, Y.; Hirose, Y.; Li, Y.; Tanaka, T. *J. Chem. Phys.* **1987**, *86*, 2375−2379. Katayama, S.; Hirokawa, Y.; Tanaka, T. *Macromolecules* **1984**, *17*, 2641−2643.

(4) Hirotsu, S. *J. Phys. Soc. Jpn.* **1987**, *56*, 233−242. Hirotsu, S. *J. Chem. Phys.* **1988**, *88*, 427−431.

(5) Asano, M.; Winnik, F. M.; Yamashita, T.; Horie, K. *Macromolecules* **1995**, *28*, 5861−5866.

(6) Mukae, K.; Sakurai, S.; Sawamura, S.; Makino, K.; Kim, S. W.; Ueda, I.; Shirahama, K. *J. Phys. Chem.* **1993**, *97*, 737−741.

(7) Otake, K.; Inomata, H.; Konno, M.; Saito, S. *Macromolecules* **1990**, *23*, 283−289.

(8) Zhu, P. W.; Napper, D. H. *J. Colloid Interface Sci.* **1996**, *177*, 343−352.

(9) Schild, H. G.; Tirrell, D. A. *J. Phys. Chem.* **1990**, *94*, 4352−4356.

(10) Eliassaf, J. *J. Appl. Polym. Sci.* **1978**, *22*, 873−874.

(11) Mukae, K.; Bae, Y. H.; Okano, T.; Kim, S. W. *Polym. J.* **1990**, *22*, 250−256. Bae, Y.; Okano, T.; Kim, S. W. *Pharmacol. Res.* **1991**, *8*, 624−628.

(12) Wolf, B. A.; Willms, M. M. *Makromol. Chem.* **1978**, *179*, 2265−2277.

(13) de Gennes, P. G. *J. Phys. Lett.* **1976**, *37*, 59−61. Brochard, F.; de Gennes, P. G. *Ferroelectrics* **1980**, *30*, 33−47.

(14) Tanaka, H.; Gubbins, K. E. *J. Chem. Phys.* **1992**, *97*, 2626−2634. Gonzalez, L.; Mo, O.; Yanez, M. *J. Chem. Phys.* **1998**, *109*, 139−150. Jorgensen, W. L.; Gao, J.; Ravimohan, C. *J. Phys. Chem.* **1985**, *89*, 3470−3473. Iosue, J. L.; Benoit, D. M.; Clay, D. C. *Chem. Phys. Lett.* **1999**, *301*, 275−280.

(15) Katz, E. D.; Lochmuller, C. H.; Scott, R. P. W. *Anal. Chem.* **1989**, *61*, 349−355.

(16) Franks, F. *Water, A Comprehensive Treatise*; Franks, F., Ed.; Plenum Press: New York, 1972; Vol. 2.

that water could strongly interact with methanol to form complexes, which has nothing to do with the presence of PNIPAM. A direct experimental observation of the complexation between water and methanol is difficult, if not impossible. Nearly all previous investigations were strongly dependent on different models and assumptions.

In the present study, we found that the conformation change of individual PNIPAM linear chains could be used as a sensitive indicator to probe the complexation between water and methanol. In an extremely dilute solution, the disturbance of PNIPAM to the water/methanol complexation can be neglected. It should be noted that the coil-to-globule transition of individual homopolymer chains itself is an important problem and has many implications in protein folding and denaturation,[22] DNA packing,[23] and the interchain interaction.[24] The experimental observation of the coil-to-globule transition of a linear polymer chain in solution without any interchain association (aggregation) is difficult and still remains a challenge even though such a transition has long been predicted[25-29] and investigated.[30-34] Most of the past studies were conducted in a kinetic fashion and focused on the temperature-induced transition. Only a few studies have dealt with the solvent-composition-induced transition in a mixture of two organic solvents.[35] It is expected that the solvent-composition-induced transition would have underlying physics different from the temperature-induced one. Using specially prepared ultralong linear PNIPAM chains, we have successfully observed the reentrant coil-to-globule-to-coil transition of individual PNIPAM chains and probed two critical compositions of cononsolvency of the water/methanol mixture.

Experimental Section

Sample Preparation. The synthesis of PNIPAM has been detailed before.[36] The resultant PNIPAM sample was carefully fractionated by six successive dissolution/precipitation cycles at 25 °C in a mixture of

extremely dried acetone and *n*-hexane. In each cycle, only the very first fraction was used for the next cycle. The PNIPAM fraction finally used had a weight average molar mass (M_w) of $\sim 2 \times 10^7$ g/mol. The complete dissolution of this PNIPAM fraction in water (1.0×10^{-4} g/mL) at 25 °C took 10 days. The solution was partially filtered through a 0.2 μm filter to remove the minor component of shorter PNIPAM chains. The remaining solution was diluted with water before a proper amount of methanol was added dropwise. The final concentration of PNIPAM in different water/methanol mixtures was kept at 5.0×10^{-7} g/mL. Such diluted solutions were further clarified with a 0.5 μm Millipore Millex-LCR filter to remove dust before the light-scattering measurement. The PNIPAM solution in pure methanol was obtained by slowly removing water before adding methanol. Such a combination of successive fractionation and filtration finally led to narrowly distributed ultralong PNIPAM chains with $M_w = 2.6 \times 10^7$ g/mol and $M_w/M_n < 1.1$. The resistivity of deionized water used was 18.3 MΩ cm.

Laser Light Scattering (LLS). A modified spectrometer (ALV/SP-125) equipped with a multi-τ digital time correlation (ALV5000) and a solid-state laser (DPSS532, output power = 400 mW at $\lambda_0 = 532$ nm) as the light source was used. In static LLS,[37,38] we were able to obtain the weight-average molar mass (M_w), the second virial coefficient A_2, and the *z*-average root-mean-square radius of gyration ($\langle R_g^2 \rangle^{1/2}$ or written as $\langle R_g \rangle$) of polymer chains in a dilute solution from the angular dependence of the excess absolute scattering intensity, known as the Rayleigh ratio $R_{vv}(q)$, by using

$$\frac{KC}{R_{vv}(q)} \approx \frac{1}{M_w}\left(1 + \frac{1}{3}\langle R_g^2 \rangle q^2\right) + 2A_2C \tag{1}$$

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.

In dynamic LLS,[39] the Laplace inversion 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 $G(\Gamma)$.[38,39] For a diffusive relaxation, Γ is related to the translational diffusion coefficient D by $(\Gamma/q^2)_{C\rightarrow 0,q\rightarrow 0} \rightarrow D$, so that $G(\Gamma)$ can be converted into a translational diffusion coefficient distribution $G(D)$ or a hydrodynamic radius distribution $f(R_h)$ via the Stokes—Einstein equation, $R_h = (k_B T/6\pi\eta)\cdot D^{-1}$, 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 ($\langle\Gamma\rangle$) or an average hydrodynamic radius $\langle R_h \rangle$. All the LLS measurements were done at 20.0 ± 0.1 °C. The details of our LLS instrumentation can be found elsewhere.[36] It is worth noting that our LLS spectrometer has an exceptional small angle range down to 6°, which is vitally important for the study of the coil state of ultralong polymer chains because a precise determination of M_w, $\langle R_g \rangle$, and $\langle R_h \rangle$ requires $q\langle R_g \rangle \ll 1$. The refractive indexes (n) of different water/methanol mixtures and the refractive index increments (dn/dC) of PNIPAM in different water/methanol mixtures were, respectively, determined by an Abbe refractometer and a novel differential refractometer.[40]

Results and Discussion

Figure 1 shows that both the refractive index (*n*) and the specific refractive index increment (dn/dC) severely deviate from a simple additivity (the dash lines). The changes of *n* and dn/dC as the methanol content $x_{methanol}$ increases can be roughly divided into three ranges. In the range of $x_{methanol} < \sim 20$ mol %, they increase with $x_{methanol}$, while in the range of $x_{methanol} >$

(17) Alam, M. K.; Callis, J. B. *Anal. Chem.* **1994**, *66*, 2293−2301. Bliesner, D. M.; Sentell, K. B. *Anal. Chem.* **1993**, *65*, 1819−1826. Guillaume, Y. C.; Guinchard, C. *Anal. Chem.* **1998**, *70*, 608−615. Zhao, Z.; Malinowski, E. R. *Anal. Chem.* **1999**, *71*, 602−608.

(18) Wakisaka, A.; Abdoul-Carime, H.; Yamamoto, Y.; Kiyozumi, Y. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 369−374.

(19) Sato, T.; Chiba, A.; Nozaki, R. *J. Chem. Phys.* **2000**, *112*, 2924−2932.

(20) Onori, G. *J. Chem. Phys.* **1987**, *87*, 1251−1255.

(21) Soper, A. K.; Finney, J. L. *Phys. Rev. Lett.* **1993**, 71, 4346−4349.

(22) Creighton, T. E. *Protein folding*; W. H. Freeman and Co.: New York, 1992.

(23) Chan, H. S.; Dill, K. A. *Phys. Today* **1993**, *46* (2), 24−32.

(24) Tsuchida, E.; Abe, K. *Adv. Polym. Sci.* **1982**, *45*, 1−119.

(25) Stockmayer, W. H. *Macromol. Chem.* **1960**, *35*, 54−74.

(26) Ptitsyn, O. B.; Kron, A. K.; Eizner, Y. Y. *J. Polym. Sci., Part C* **1968**, *16*, 3509−3517.

(27) de Gennes, P. G. *J. Phys. Lett.* **1975**, *36*, 55−57.

(28) Yamakawa, H. *Modern Theory of Polymer Solutions*; Harper & Row: New York, 1971. Yamakawa, H. *Macromolecules* **1993**, *26*, 5061−5066.

(29) Grosberg, A. Y.; Khokhlov, A. R. *Statistical Physics of Macromolecules*; AIP Press: Woodbury, NY, 1984.

(30) Post, C. B.; Zimm, B. H. *Biopolymers* **1979**, *18*, 1487−1501. Post, C. B.; Zimm, B. H. *Biopolymers* **1982**, *21*, 2123−2137.

(31) Sun, S. T.; Nishio, I.; Swislow, G.; Tanaka, T. *J. Chem. Phys.* **1980**, *73*, 5971−5975.

(32) Chu, B.; Park, I. H.; Wang, Q. W.; Wu, C. *Macromolecules* **1987**, *20*, 2833−2840.

(33) Kubota, K.; Fujishige, S.; Ando, I. *J. Phys. Chem.* **1990**, *94*, 5154−5158.

(34) Nakata, M. *Phys. Rev. E* **1995**, *51*, 5770−5775.

(35) Nakata, M.; Nakagawa, T. *Phys. Rev. E* **1997**, *56*, 3338−3345 and the reference therein.

(36) Wu, C.; Zhou, S. Q. *Macromolecules* **1995**, *28*, 5388−5390. Wu, C.; Zhou, S. Q. *Phys. Rev. Lett.* **1996**, *77*, 3053−3055. Wu, C.; Wang, X. *Phys. Rev. Lett.* **1998**, *80*, 4092−4094. Wang, X.; Qiu, X.; Wu, C. *Macromolecules* **1998**, *31*, 2972−2976.

(37) Zimm, B. H. *J. Chem. Phys.* **1948**, *16*, 1099−1116.

(38) Chu, B. *Laser Light Scattering*, 2nd ed.; Academic Press: New York, 1991.

(39) Berne, B.; Pecora, R. *Dynamic Light Scattering*; Plenum Press: New York, 1976.

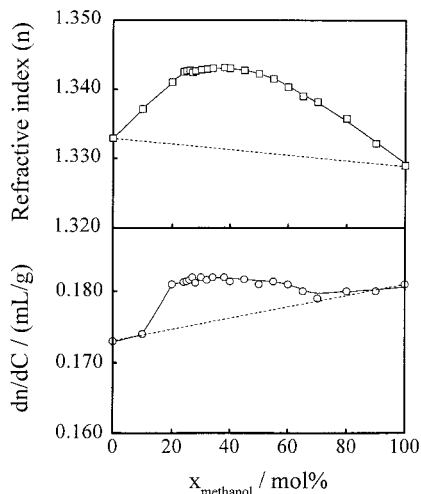(40) Wu, C.; Xia, K. Q. *Rev. Sci. Instrum.* **1994**, *65*, 587−590.

Figure 1. Methanol molar fraction ($x_{methanol}$) dependence of refractive index (n) of water/methanol mixtures and the corresponding specific refractive index increments (dn/dC) of PNIPAM, where $x_{methanol}$ is defined as [methanol]/([methanol] + [H_2O]).
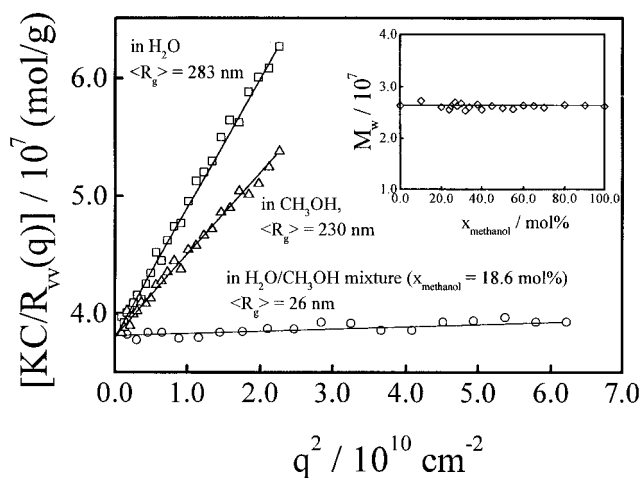

Figure 2. Angular dependence of excess scattered light intensity $KC/R_{vv}(q)$ of PNIPAM chains in water, water/methanol mixture, and methanol, respectively. The inset is the molar methanol fraction ($x_{methanol}$) dependence of the relative scattering intensity of the PNIPAM solution; I and I_r are the scattering intensity and reference intensity, respectively.

50 mol %, they decrease as $x_{methanol}$ increases. In the middle range ~20−50 mol %, there is nearly no change in n and dn/dC. The nonideal behavior indicates a strong interaction between water and methanol. Assuming that the water/methanol complexes have a higher refractive index, we could explain the change of n as $x_{methanol}$ increases. In the low and high $x_{methanol}$ range, the mixture could be respectively regarded as a binary mixture of "water and the complexes" and "methanol and the complexes", while in the middle range, different water/methanol complexes coexist. In the low $x_{methanol}$ range, the addition of methanol leads to the formation of more complexes with a higher refractive index, so that the overall n increases, while in the high $x_{methanol}$ range, the complexation between water and methanol is saturated so that further addition of methanol could only result in free methanol, which explains the decrease of the overall n. The effect on dn/dC could be attributed to the dehydration of PNIPAM. We will discuss this point later.

Figure 2 shows that in comparison with PNIPAM in pure water and pure methanol, the slope of $KC/R_{vv}(q)$ versus q^2 decreases sharply. This corresponds to a more than 10 times decrease in $\langle R_g \rangle$ on the basis of eq 1, clearly showing the
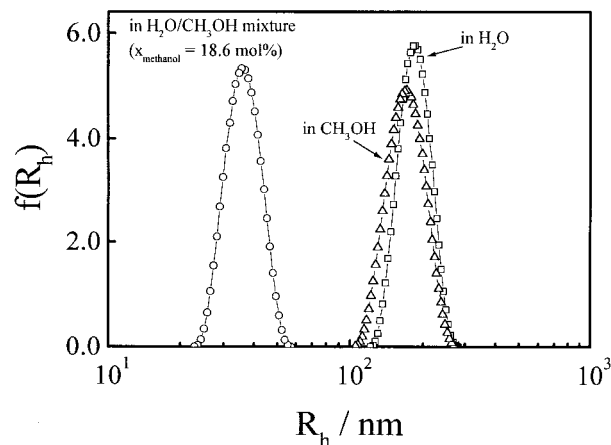

Figure 3. Typical hydrodynamic radius distributions $f(R_h)$ of PNIPAM in water, water/methanol mixture, and methanol, respectively.

collapse of the chains in the water/methanol mixture. The remarkable point is that despite a sharp decrease in the slope, there is no change in the intercept, i.e., no change in M_w, as shown in the inset of Figure 2. Therefore, there is no interchain aggregation in the reentrant transition and the conformation change involves only individual PNIPAM chains, because for a given concentration (g/mL), $R_{vv}(q)$ is proportional to the square of molar mass, i.e., $R \propto M_w \propto nM^2$, very sensitive to the interchain aggregation. In principle, such a plot should be shown in every reported study of the coil-to-globule transition of individual polymer chains. It should be stated that for PNIPAM in each given mixture, the scattering light intensity is independent of the standing time, indicating that such formed single chain globules are stable in solution.

Figure 3 shows that the PNIPAM sample used is narrowly distributed with a relative width $\mu_2/\langle\Gamma\rangle^2$ in the range ~0.01−0.02. The polydispersity index (M_w/M_n) estimated from $1 + 4(\mu_2/\langle\Gamma\rangle^2)$ is less than 1.1.[41] Note that the x-axis in Figure 3 is logarithmic. The difference in $f(R_h)$ shows that the PNIPAM chains have a more extended chain conformation in water than in methanol, indicating that water is a better solvent than methanol for PNIPAM at 20 °C.[42] The large shift of $f(R_h)$ to the left clearly reveals the collapse of the chains in the water/methanol mixture. From each $f(R_h)$, we can calculate an average hydrodynamic radius $\langle R_h \rangle$ defined as $\int_0^\infty f(R_h)R_h \, dR_h$.

Figure 4 shows a clear reentrant coil-to-globule-to-coil transition in terms of both $\langle R_g \rangle$ and $\langle R_h \rangle$. It should be stated that even in the fully collapsed globule state, $\langle R_g \rangle$ and $\langle R_h \rangle$ were independent of the standing time; namely, each point in Figure 4 represents a stable value. The sharp drops of $\langle R_g \rangle$ and $\langle R_h \rangle$ at $x_{methanol} \sim 17$ mol % show the collapse of the chains. The addition of methanol in the range 20−40 mol % has little effect on the chain conformation in the collapsed state. Further addition of methanol finally leads to the rewinding of the globule back into the coil conformation, i.e., the globule-to-coil transition, at $x_{methanol} \sim 50$ mol %. Note that $\langle R_g \rangle$ decreases more than 10 times from ~280 nm in pure water to ~26 nm in the methanol/water mixture. Also note that both $\langle R_g \rangle$ and $\langle R_h \rangle$ are independent of the methanol content when $x_{methanol} > 50$ mol %. This reentrant transition of individual PNIPAM chains is well correlated to the swelling−shrinking−swelling of PNIPAM gels.[1,4−8] A careful examination of Figure 4 shows that in the coil-to-globule transition, $\langle R_h \rangle$ drops slower than $\langle R_g \rangle$, while in the rewinding process, $\langle R_g \rangle$ increases much faster than $\langle R_h \rangle$.

(41) Chu, B.; Wang, Z.; Yu, J. *Macromolecules* **1991**, *24*, 6832−6838.
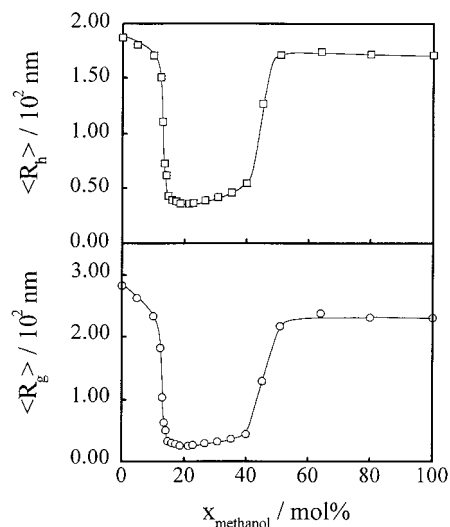(42) Fujishige, S. *Polym. J.* **1987**, *19*, 297−300.

Figure 4. Methanol molar fraction (x_{methanol}) dependence of the average radius of gyration $\langle R_g \rangle$ and average hydrodynamic radius $\langle R_h \rangle$ of PNIPAM.
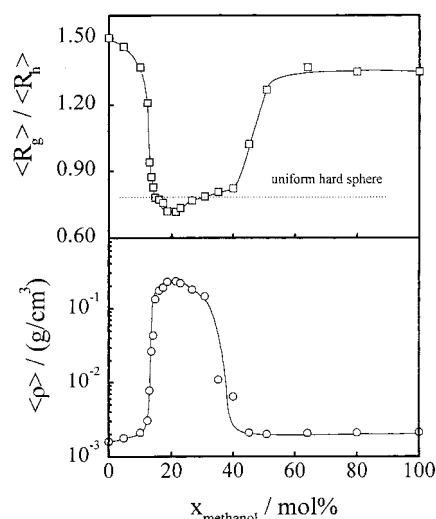

Figure 5. Methanol molar fraction (x_{methanol}) dependence of $\langle R_g \rangle / \langle R_h \rangle$ and average chain density $\langle \rho \rangle$ of PNIPAM chains in methanol/water mixtures.

These differences can be attributed to the fact that dynamic $\langle R_h \rangle$ is sensitive to the hydrodynamic draining, while static $\langle R_g \rangle$ depends only on the spatial chain density distribution. Such a reentrant coil-to-globule-to-coil transition can be better viewed in terms of the ratio of $\langle R_g \rangle / \langle R_h \rangle$.

Figure 5 shows that in pure water, $\langle R_g \rangle / \langle R_h \rangle \sim 1.5$, indicating that the PNIPAM chain in water has a coil conformation. The sharp decrease of $\langle R_g \rangle / \langle R_h \rangle$ as x_{methanol} increases is due to the collapse of the chains. Note that at $x_{\text{methanol}} \sim 20$ mol %, $\langle R_g \rangle / \langle R_h \rangle \sim 0.72$, less than 0.774 (the dotted line) predicted for a uniform hard sphere.[43,44] The low value of $\langle R_g \rangle / \langle R_h \rangle$ can be associated with the molten globule state in which small loops are formed on the periphery of the globule due to the chain folding in the collapsing process.[36] These small loops are presumably nondraining so that they lead to a larger $\langle R_h \rangle$, but have less effect on $\langle R_g \rangle$ because of their insignificant masses. When $x_{\text{methanol}} > 50$ mol %, $\langle R_g \rangle / \langle R_h \rangle$ remains a constant (~ 1.35), just as in pure methanol. Note that PNIPAM in pure

methanol has a lower value of $\langle R_g \rangle / \langle R_h \rangle$ than in pure water, further revealing that water is a better solvent for PNIPAM at 20 °C. Using $\langle \rho \rangle = M_w / [N_A (4/3) \pi (\langle R_h \rangle^3)]$, we were able to estimate the average chain density $\langle \rho \rangle$. As shown in Figure 5, $\langle \rho \rangle$ increases very quickly when x_{methanol} reaches 17 mol % but drops at $x_{\text{methanol}} > 50$ mol %. Moreover, $\langle \rho \rangle$ has a maximum value of ~ 0.23 g/cm^3, less than ~ 0.4 g/cm^3 predicted on the basis of a space-filling model.[45] It shows that even in the fully collapsed state, the globule still contains $\sim 70-80\%$ solvents inside its hydrodynamic volume.

The reentrant coil-to-globule-to-coil transition of individual PNIPAM chains clearly shows that the water/methanol mixtures in the range $17-50$ mol % are poor solvents for PNIPAM even though water and methanol individually are good solvents. Note that in this study, the PNIPAM concentration was so low, on average, that each N-isopropylacrylamide monomer unit was surrounded by $\sim 10^7$ solvent molecules. The existence of such a trace amount of PNIPAM should have a negligible effect on the water/methanol interaction. In a solution of PNIPAM in the water/methanol mixture, there exist at least three kinds of intermolecular interactions, namely, the interactions between water and PNIPAM, between methanol and PNIPAM, and between water and methanol. Our results suggest that the water/methanol interaction is stronger than the other two interactions. Recent spectroscopic and high-performance liquid chromatographic studies on the water/methanol complexation showed that when the methanol content was low, one methanol could complex with a number of water molecules, while in the high methanol content, the ratio became one to one.[15,17-19] These water/methanol complexes were thought to coexist with free water or free methanol, depending on the methanol content. Note that all the results related to the water/methanol complexation were interpreted in terms of certain kinds of models or assumptions. Up to now, we were still not able to directly detect or probe such a complexation, even if it looks simple.

Figures 4 and 5 reveal that $x_{\text{methanol}} \sim 17$ mol % and ~ 50 mol %, i.e., $[\text{H}_2\text{O}]/[\text{CH}_3\text{OH}] \sim 5:1$ and $\sim 1:1$, are two critical solvent compositions, corresponding to two sharp transitions. Note that the solubilization of PNIPAM in water or methanol is due to the association of water or methanol molecules on the polymer chain, presumably on the carbonyl and amide groups. The PNIPAM concentration is so low that even a small amount of uncomplexed free water or methanol would be sufficient to solubilize the PNIPAM chains and keep them in the coil state. However, we should not forget that most of the free water or methanol molecules, if they exist, are sitting in the ocean of solvent due to the entropy effect. The abrupt chain collapse and rewinding at these two critical compositions must correspond to a nearly complete complexation between water and methanol. Otherwise, we would not be able to observe two such sharp transitions in Figures 4 and 5. Therefore, the chain conformation change resembles the color change of an indicator in a titration experiment. These two critical compositions suggest the formation of $(\text{H}_2\text{O})_{5m}(\text{CH}_3\text{OH})_m$ and $(\text{H}_2\text{O})_m(\text{CH}_3\text{OH})_m$ complexes, respectively, at low and high methanol content, which are different from those reported before.[15,17,18]

Our explanation is as follows. When $x_{\text{methanol}} < 17$ mol %, methanol are not sufficient to complex with all water molecules to form $(\text{H}_2\text{O})_{5m}(\text{CH}_3\text{OH})_m$, so that some of water molecules are free in the mixture. It is these free water molecules that solubilize the trace amount of PNIPAM. As the methanol content increases, more $(\text{CH}_3\text{OH})_m(\text{H}_2\text{O})_{5m}$ complexes are

(43) Burchard, W.; Schmidt, M.; Stockmayer, W. H. *Macromolecules* **1980**, *13*, 1265–1272.
(44) Acksasu, A. Z.; Han, C. C. *Macromolecules* **1979**, *12*, 276–280.

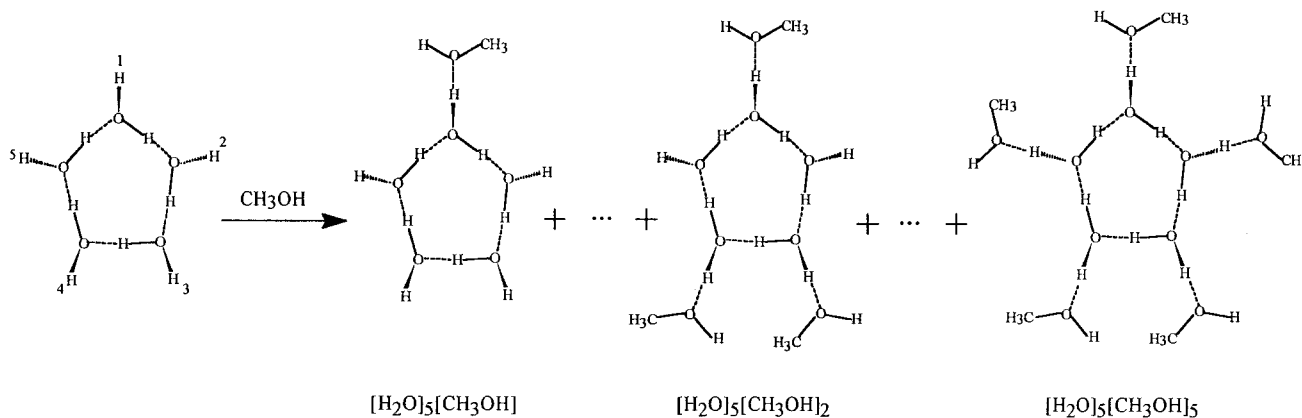(45) Marchetti, M.; Prager, S.; Cussler, E. L. *Macromolecules* **1990**, *23*, 3445–3450.

Figure 6. Schematic of the pentagon structure of pure water and its possible complexation with different numbers of methanol molecules.

formed, and the solvent quality decreases, resulting in a slight contraction of the PNIPAM chains in the range 0−17 mol %. When $x_{methanol}$ reaches ∼17 mol %, the formation of $(H_2O)_{5m}(CH_3OH)_m$ leads to a nearly complete dehydration of PNIPAM resulting in a sharp coil-to-globule transition. The fact that further addition of methanol in the range $x_{methanol} >$ ∼50 mol % has no effect on the conformation suggests that the maximum methanol/water ratio is one. When $x_{methanol} >$ 50 mol %, the PNIPAM chains are actually soluble in the excess of methanol molecules free in the mixture. Nearly no conformation change in the middle range ∼17−50 mol % indicates that there should exist no free water or methanol. The only possibility would be a gradual change from $(H_2O)_{5m}(CH_3OH)_m$ to $(H_2O)_m(CH_3OH)_m$ via different intermediates $(H_2O)_m(CH_3OH)_n$ with $m/n = 5/2$, 5/3, or 5/4.

Molecular dynamics calculations[46] and some experiments[47] showed that water predominately exists in the form of pentagon via five intermolecular hydrogen bonds, as shown in Figure 6. Our results experimentally support such a structure. In pure water, five hydrogens are free from the ring formation. It is these five hydrogens that are capable of forming the complexation with 1 to 5 methanol molecules via hydrogen bonding, resulting in different water/methanol complexes. Note that hydrogen 1 is out of the plane defined by the other four hydrogens. Therefore, we can speculate that it is this hydrogen that complexes with one methanol molecule when $x_{methanol}$ is low. As the methanol content increases, the other four hydrogen start to complex with methanol to form a mixture of different complexes. The maximum complexation number could only be one to one on the basis of this model. Our results support such a complexation mechanism.

Finally, it should be stated that a similar reentrant phenomenon of PNIPAM has also been observed in the mixture of water with other water-miscible polar solvents, such as acetone,[1] dioxane,[1] dimethyl sulfoxide,[1,3] tetrahydrofuran,[1,2] and other alcohols.[1,5−8] More evidence about the complexation of water with these solvents is appearing.[48] In a parallel study, we found that hydrogen peroxide could significantly depress the transition temperature of PNIPAM in water even though it has no hydrophobic groups. Therefore, it suggests that the stronger

hydrogen bonding between water and these polar solvents is responsible for the reentrant transition of PNIPAM. Further experimental and theoretical studies are certainly required to explore the complexation between water and these polar solvents.

Conclusion

For poly(*N*-isopropylacrylamide) (PNIPAM), a proper mixture of two good solvents, water and methanol, can lead to a poor solvent. We have shown, *for the first time,* that linear PNIPAM homopolymer chains can collapse into individual stable single-chain globules if the methanol content ($x_{methanol}$) is in the range ∼17−50 mol %. The dissolution of PNIPAM in the range of $x_{methanol} <$ ∼17 mol % and $x_{methanol} >$ ∼50 mol % makes the chain conformation transition become a reentrant one. Our results suggest that this reentrant coil-to-globule-to-coil transition could be attributed to the formation of different water/methanol complexes which are poor solvents for PNIPAM. Moreover, we have shown that the conformation change of individual PNIPAM chains in the water/methanol mixture can be used as a very sensitive indicator to quantitatively probe the critical solvent composition and the complexation between water and methanol. Our results suggest that in the range ∼17−50 mol %, the composition of the complexes gradually changes from $(H_2O)_{5m}(CH_3OH)_m$ to $(H_2O)_m(CH_3OH)_m$ via different intermediates $(H_2O)_m(CH_3OH)_n$ with $m/n = 5/2$, 5/3, or 5/4, as $x_{methanol}$ increases, which supports the hypothesis that water predominately exists in the pentagon form.

(46) Rahman, A.; Stillinger, F. H. *J. Am. Chem. Soc.* **1973**, *95*, 7943−7948. Speedy, R. J.; Madura, J. D.; Jorgensen, W. J. **1987**, *91*, 909−913. Corongiu, G.; Clementi, E. *J. Chem. Phys.* **1993**, *98*, 2241−2249.
(47) Liu, K.; Brown, M. G.; Cruzan, J. D.; Saykally, R. J. *Science* **1996**, *271*, 62−64.

(48) Feakins, D.; Bates, F. M.; Waghorne, W. E.; Lawrence, K. G. *J. Chem. Soc., Faraday Trans.* **1993**, *89*, 3381−3388. Murthy, S. S. N. *J. Phys. Chem. A* **1999**, *103*, 7927−7937. Laria, D.; Skaf, M. S. *J. Chem. Phys.* **1999**, *111*, 300−309. Dawber, J. G. *J. Chem. Soc., Faraday Trans.* **1990,** *86*, 287−291. Muller, N.; Simon, P. *J. Phys. Chem.* **1967**, *71*, 568−572. Burneau, A.; Corset, J. *J. Phys. Chem.* **1972**, *76*, 449−452.