Fluorescence Studies of Dansyl-Labeled Poly(*N*-isopropylacrylamide) Gels and Polymers in Mixed Water/Methanol Solutions

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ABSTRACT: The photophysical properties of solutions of dansyl [5-(dimethylamino)naphthalenyl-1-sulfonyl] labeled poly(*N*-isopropylacrylamide) (PNIPAM-Da; M_v 220 000; label content, 0.06 molar %) were examined in mixtures of water and methanol in order to investigate the phenomenon of cononsolvency exhibited by the PNIPAM/water/methanol ternary system. They were compared to the photophysical properties of dansyl-labeled poly(*N*-isopropylacrylamide) gels (cross-linker, *N,N'*-methylenebis(acrylamide); cross-linker content, 1.2 molar %; probe content, 0.06 molar %) equilibrated in mixtures of methanol and water. The swelling of these gels decreases abruptly in aqueous solutions containing 7–25 mol % methanol and increases gradually in systems with a higher methanol concentration. Steady-state emission and excitation spectra, fluorescence decays, and fluorescence polarization were recorded at 20 °C from polymer solutions and from equilibrated gels. Shifts in the wavelength of maximum emission and changes in the fluorescence lifetimes and in the rotational diffusion coefficients of the probe were correlated to the macroscopic changes in swelling volume.

Introduction

In 1949 Katchalsky described a synthetic "contractile system" exhibiting on a macroscopic scale contractions and dilatations which he believed to be akin to the motions of biological systems such as the muscles.[1] He showed that a crosslinked poly(methacrylic acid) gel undergoes rapid and reversible swelling/shrinking processes by successive additions of alkali and acids. This account laid the foundations for a fast-growing field, which encompasses the theory of gels, the chemical synthesis and physics of gels, and the technology associated with gel-based stimuli–responsive devices.[2] A battery of techniques were developed to monitor phase transitions by measuring gel properties such as volume,[3] shear modulus,[4] and osmotic compressibility[5] as a function of temperature and additives. Such studies reveal the multiple facets of the phenomenon and emphasize the need for a better understanding of the molecular interactions at play. Diffusion coefficients within gel networks were determined by light scattering[6] and small-angle X-ray scattering.[7] Dynamics of probe particles within gels were investigated using photon correlation spectroscopy.[8] NMR spectroscopy proved to be a powerful technique to monitor the dynamics of the phase transitions and to elucidate changes in the local environment of the polymer chains and the solvent molecules.[9,10] The critical phenomena of the volume phase transition were studied by calorimetry,[11] friction measurements,[12] dynamic light scattering,[13] and small-angle neutron scattering.[14]

Spectroscopic techniques which require the use of labels covalently attached to gels provide another "insider" look at the phase transition. EPR-active labels have been used in dextran-based hydrogels.[15] Fluorescent labels were attached to gels to monitor the degree of crosslinking[16] and the permeability of gels to various solvents.[17] Recently several groups have reported applications of fluorescence spectroscopy to study volume phase transitions in gels. Building on the experience they gained during early studies of crosslinked polystyrene gels,[18] Horie *et al.* have explored the applications of fluorescence depolarization to assess the dynamic fluctuations within poly(acrylamide) gels as they undergo volume phase transitions.[19–21] By performing the same fluorescence measurements with solutions of the corresponding labeled polymer, it becomes possible also to study the consequences of crosslinking on the dynamics of the polymer chains and on their interactions with solvent molecules.[16]

N-Isopropylacrylamide gels are known to undergo a volume phase collapse in water around 34 °C, in response to infinitesimal changes in temperatures.[2,22–25] The gels also undergo discontinuous volume phase transitions between swollen and collapsed states in response to changes in solvent composition. For example, they exist in a swollen state both in water and in methanol but are collapsed when equilibrated in certain water/methanol mixtures. The phase diagram contains a closed-loop two-phase region with upper and lower critical points. The theoretical implications of this unusual situation have been treated by Amiya and coworkers[26] and by Hirotsu.[27] This tendency of PNIPAM gels has been exploited also in the controlled-release of alcohol-soluble drugs.[28] It is not clear why the gel shows such a curious behavior, although it can be interpreted qualitatively in terms of the attractive interactions between water and methanol molecules, on the one hand, and hydrogen bonding between polymer chains, on the other.

In this context it is appropriate to study also the phase transitions exhibited by free poly(*N*-isopropylacrylamide) chains dissolved in water and in mixed solvents. The polymer is soluble in cold water but

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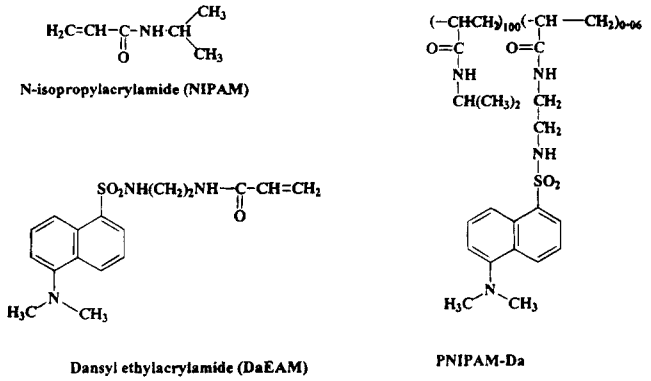

Figure 1. Chemical structures of the monomers and polymers employed in this study.

separates from solution upon heating, with a lower critical solution temperature (LCST) of 31 °C.[29] The solubility of PNIPAM in aqueous solutions is sensitive not only to changes in temperature but also to the solvent composition, in particular to solutes that modify the water structure. In analogy to the gel behavior, the polymer is soluble in cold water and in methanol but is insoluble in certain mixtures of these solvents.[30,31] From experimental evidence gathered by microcalorimetry,[24] EPR spectroscopy,[32,33] and fluorescence spectroscopy,[34,35] it is assumed that the most likely mechanism for the LCST of PNIPAM in mixed solvent involves a ternary complex between the polymer chain and the two solvents, with structural changes occurring mainly at the outer solvent layers of a highly ordered structure present already in the dissolved phase.

We present here a comparative study of the solvent-induced volume transition of fluorescently labeled PNIPAM gels and of the corresponding co-nonsolvency phenomenon exhibited by labeled PNIPAM in the same solvent mixtures. We have used the same chromophore, *N,N*-(dimethylamino)naphthalenesulfonamide (Da, dansyl group), to label the polymer and the gel. The fluorescence of the labeled gels and polymers was monitored as a function of solvent composition by steady-state and time-resolved spectroscopy. Steady-state fluorescence spectra were recorded also under polarized light excitation. The fluorescence spectroscopy of the dansyl group yields information on the micropolarity and microviscosity of the immediate environment sensed by the probe.[36] Our experiments reveal differences in local microviscosity during the solvent-induced collapse for probes attached either to gels or to polymer chains. The implications of this unexpected result are discussed.

Experimental Section

Materials. Reagent-grade solvents and distilled water were employed. *N*-Isopropylacrylamide (Tokyo Kasei, reagent grade) was purified by precipitation with *n*-hexane from a solution in benzene. *N*-[2-[[5-(Dimethylamino)naphthalene-1-sulfonyl]amino]ethyl]-2-propenamide (Dansyl ethylacrylamide, DaEAM; Figure 1) was prepared by reaction of acryloyl chloride with *N*-(2-aminoethyl)-5-(dimethylamino)-1-naphthalenesulfonamide.[17]

Preparation of Poly(*N*-isopropylacrylamide) (PNIPAM). *N*-Isopropylacrylamide (5.0 g, 0.044 mol) was dissolved in 2-methyl-2-propanol (25 mL) at 70 °C under nitrogen. AIBN (30 mg) in 2-methyl-2-propanol (1 mL) was added at once. The solution was stirred for 20 h at 70 °C. It was cooled to room temperature. The solvent was evaporated. The residual material was dissolved in THF (10 mL) and precipitated into *n*-hexane. The isolated polymer (3.7 g) was purified further by precipitation of a solution in methanol (100 mL) into dry diethyl ether.

Preparation of Dansyl-Labeled PNIPAM (PNIPAM-Da). A solution of *N*-isopropylacrylamide (5.0 g, 0.044 mol) and DaEAM (8.8 mg, 0.025 mmol) in dioxane (25 mL) was heated to 70 °C. AIBN (30 mg) in dioxane (1 mL) was added at once. The solution was stirred at 70 °C for 20 h. It was cooled to room temperature. The solvent was evaporated. The residual material was dissolved in THF (10 mL) and reprecipitated into *n*-hexane. The isolated polymer (3.1 g) was purified further by precipitation of a solution in methanol (30 mL) into dry diethyl ether. The purity of the polymer was ascertained by GPC analysis with a chromatography system equipped with UV and refractive index detectors in tandem. The molecular weight of the polymer was estimated from the intrinsic viscosity, $[\eta]$, of the polymer in THF, using the viscometric relationship: $[\eta] = 9.59 \times 10^{-3} M_v^{0.65}$ cm^3 g^{-1}.[38] GPC analysis of the polymers in THF yields M_w and M_n values, based on polystyrene standards (M_v = 220 000, M_n 55 000, M_w 130 000, M_w/M_n 2.4).

Preparation of Dansyl-Labeled PNIPAM Gels. A solution of *N*-isopropylacrylamide (7.9 g, 0.070 mol), *N,N'*-methylenebis(acrylamide) (BIS; 0.13 g, 0.86 mmol) and DaEAM (0.14 g, 0.4 mmol) in DMF/water (2/3, v/v) was flushed with nitrogen for 30 min. Ammonium persulfate (0.020 g, 0.088 mmol) and tetramethylethylenediamine (TEMED; 0.04 mL, 0.27 mmol) were added to this solution which was then made up to 100 mL with DMF/water (2/3, v/v). After stirring for 30 min, the solution was flushed again with nitrogen. It was transferred to glass tubes (8.0 mm i.d.). The tubes containing this pregel solution were kept at room temperature for several days to ensure completion of the polymerization. The gels were then taken out of the tubes and cut in 17-mm-long cylinders. They were rinsed with water several times.

Instrumentation. UV spectra were recorded with a Jasco-660 UV–vis spectrophotometer. Steady-state fluorescence spectra were measured with a Hitachi 859 fluorescence spectrometer. Fluorescence decays were measured with a Horiba NAES-100 single photon-counting apparatus equipped with a hydrogen pulse lamp (half-width of the pulse: 1.5–2.5 ns). Toshiba Y-47 and KL-53 filters were used. Gel permeation chromatography (GPC) was performed with a Waters 590 programmable HPLC system equipped with a refractive index detector (eluent, THF; flow rate, 1.00 mL min^{-1}). The weight and number molecular weight averages were determined from a calibration curve established with polystyrene standards. Solution viscosities in THF were measured at 25 °C with an Ubbelohde viscometer.

Cloud-Point Measurements. The cloud points of PNIPAM and of PNIPAM-Da solutions in water were determined by spectrophotometric detection of the changes in the turbidity of solutions heated at a constant rate (0.1 °C min^{-1}) and irradiated at 500 nm. Quartz cells containing the solutions were placed in the thermostated cell holder of the fluorescence spectrophotometer. The intensity of the scattered light was measured in the right-angle configuration. The points of sharp turbidity increase in plots of scattered-light intensity vs temperature were taken as the cloud-point temperatures.

Swelling Equilibrium Measurements. The water-washed gels were immersed in methanol/water mixtures of varying methanol content and kept in a thermostated bath (20 °C) for over 2 weeks to ensure equilibration. The diameter, *d*, of the equilibrated gels was measured with a vernier micrometer. The swelling ratio $V/V_0 = (d/d_0)^3$ of the gels was calculated from the ratio of the equilibrium diameter to the diameter at the time of preparation, d_0 = 8.0 mm.

Fluorescence Measurements. Steady-state spectra and fluorescence decays were recorded at 20 °C with an excitation wavelength of 330 nm. The labeled polymer solutions were placed in 1-mm-thin quartz cells, and their emission was recorded in the front-face geometry. The gels were placed in rectangular quartz cells containing a methanol/water mixture of composition identical to that used for gel preequilibration. Fluorescence spectra of the gels in the swollen state (8–24 (v/v) MeOH and 68–96 (v/v)MeOH) were measured in the right-angle geometry, while spectra of the collapsed gels (24–

Table 1. Composition and Properties of the Dansyl-Labeled Polymer and Gel

	monomer composition, mmol (mol %)			T (transition) (°C)
	NIPAM	DaEAM	MBAM	
PNIPAM-Da	44	0.025 (0.056)		32[a]
PNIPAM gel	70	0.040 (0.56)	0.86 (1.2)	34[b]

[a] Lower critical solution temperature (in water). [b] Volume phase transition temperature (in water).

64 (v/v)MeOH) were recorded in the front-face configuration. Samples for analysis were not degassed. Fluorescence decays were monitored at 530 nm. The transient decays were analyzed with a Marquardt method of convolution of the system response function. The parameters of the fitting function were varied until the best least-squares fit with the experimental data was achieved. Fluorescence anisotropies (r) were determined with a Hitachi 650 fluorimeter equipped with polarizers in the right-angle configuration. The value of r was calculated from the relationship (eq 1):

$$r = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH}) \quad (1)$$

where $G = I_{HV}/I_{HH}$ is an instrumental correction factor and I_{VV}, I_{VH}, I_{HV}, and I_{HH} refer to the resultant emission intensities polarized in the vertical or horizontal detection planes (second subindex) upon excitation with either vertically or horizontally polarized light (first subindex). The values for r showed no wavelength dependence. They were averaged for the 480–580 nm emission range.

Results and Discussion

Preparation and Properties of the Labeled Gels and Polymers. Although the swelling properties of the gels depend on the molar ratio of monomer to crosslinker in the pregel solution, the macroscopic properties of NIPAM gels are also extremely sensitive to the preparation procedure. To ensure reproducibility, it is necessary to control tightly parameters such as the purity of the monomer, the reaction time, the temperature, the oxygen concentration, and the initiator type and concentration.[37] Even mold geometry becomes important in the case of slightly charged gels. Aware of this caveat, we prepared all gels under identical conditions. A pregel solution in a DMF/water mixture containing all the reagents was kept at room temperature for 30 min before being poured into capillary glass tubes. The tubes containing this pregel solution were kept at room temperature for several days to ensure complete polymerization. Then, the gels were taken out of the tubes, cut into cylinders, and rinsed thoroughly with water. Fluorescently labeled gels were obtained by adding to the pregel solution the dansylated monomer, DaEAM (Figure 1). The monomer feed ratio of DaEAM to NIPAM was 0.06 mol % (Table 1).

The degree of swelling of PNIPAM-Da gels equilibrated in a series of MeOH/water mixtures was determined and used to build the phase diagram presented in Figure 2. The swelling ratios V/V_0, where V and V_0 are the volumes of the gels after equilibration and immediately after preparation, respectively, were calculated from the measured sample diameters, d and d_0 after swelling and as prepared, respectively. The changes in the total length of the tubes were measured as well in order to ascertain that the volume changes were isotropic. The dependence of the swelling on the solvent composition is shown in Figure 2 and is similar to that reported previously.[38b,39]

Linear polymers were prepared by free-radical polymerization of NIPAM in dioxane. The dansyl label was incorporated by adding to the polymerization

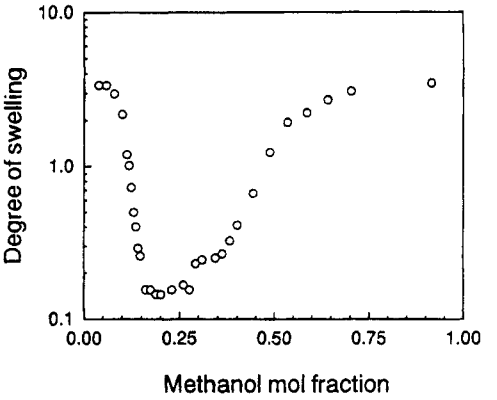

Figure 2. Degree of swelling (V/V_0) of the dansyl-labeled gels as a function of solvent composition in MeOH/water mixtures. Temperature: 20 °C.

mixture 0.06 mol %, based on NIPAM, of the dansyl-labeled comonomer DaEAM (Figure 1). The purity of the polymer was ascertained by GPC analysis with a chromatography system equipped with UV and refractive index detectors in tandem. The amount of label attached to the polymer determined by UV analysis of solutions in methanol corresponds closely to the monomer feed ratio in the polymerization mixture. The molecular weight of the polymer was estimated from the intrinsic viscosity and GPC measurements (see the Experimental Section).

The phase diagrams of PNIPAM and PNIPAM-Da in water/methanol mixtures were determined by turbidity measurements. The diagrams for the two polymers were identical, confirming that the incorporation of small amounts of label along the polymer chain does not affect significantly the macroscopic solution properties of PNIPAM. The phase diagrams for PNIPAM and PNIPAM-Da in methanol/water mixtures were similar to those reported previously:[30,31] (i) For methanol molar fractions (x_M) lower than 0.05, the LCST is hardly affected; (ii) for MeOH molar fractions $0.05 < x_M < 0.35$, the LCST decreases sharply from 32 °C in pure water to a minimum value of −7.5 °C; (iii) for $0.35 < x_M < 0.45$ the LCST increases sharply to reach a value of 14.5 °C; (iv) for $x_M > 0.45$ (64.4, v/v) it becomes impossible to detect an LCST; the solution remains clear over the entire temperature range tested (−10 to +40 °C).

Spectroscopy of the Labeled Polymers and Gels. Steady-State Fluorescence. The fluorescence spectroscopy of dansyl derivatives has been studied extensively. It is relatively insensitive to quenching by oxygen and trace impurities. The absorption maximum is essentially independent of the medium (350 nm). We confirmed that attachment of the chromophore to a PNIPAM chain does not alter this property by recording the absorption spectra of PNIPAM-Da in various methanol/water mixtures or the fluorescence excitation spectra of labeled gels equilibrated in the same solvent mixtures.

The wavelength of maximum emission, in contrast, exhibits a strong and well-defined dependence on the polarity of the probe environment.[36] We monitored the shift of λ_{em} as a function of solvent composition in the case of PNIPAM-Da gels, the linear polymer, PNIPAM-Da, and the model compound DaEAM (Figure 3). The labeled gels and the labeled polymer exhibited similar trends. The value of λ_{em} decreases sharply over the narrow methanol concentration range $0.05 < x_M < 0.15$ corresponding to the collapse of the gels and of the polymer, and the emission maximum drops from 545

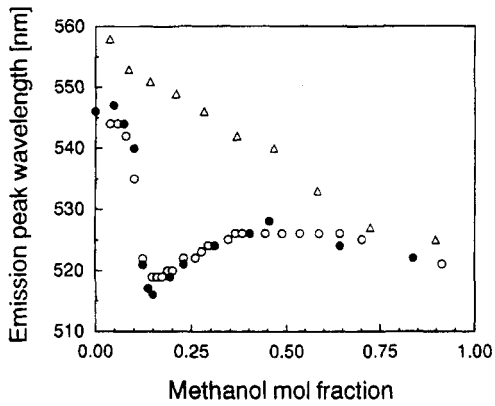

Figure 3. Changes in the wavelength of maximum emission of the PNIPAM-Da (full circles), the PNIPAM-Da gel (open circles), and the model compound DaEAM (open triangles) as a function of solvent composition in MeOH/water mixtures. Temperature: 20 °C. λ_{exc} = 330 nm.

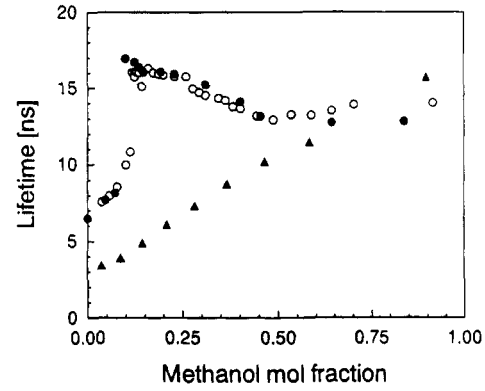

Figure 4. Changes in the fluorescence lifetimes of the dansyl label as a function of solvent composition in MeOH/water mixtures for PNIPAM-Da (full circles), PNIPAM-Da gels (open circles), and the model compound DaEAM (full triangles). Temperature: 20 °C. λ_{exc} = 330 nm; λ_{em} = 530 nm.

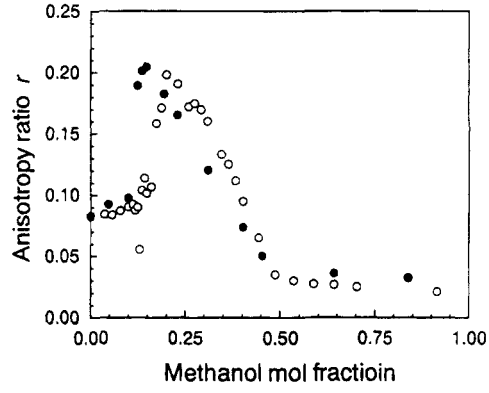

Figure 5. Changes in fluorescence anisotropy ratio (r) as a function of solvent composition in MeOH/water mixtures for PNIPAM-Da (full circles) and PNIAPM-Da gels (open circles). Temperature: 20 °C. λ_{exc} = 330 nm; λ_{em} = 480–620 nm.

to 515 nm, a value typical for dansyl groups in either acetone (513 nm) or ethanol (516 nm). Further minor perturbations in the emission of the labeled gels and polymer were observed in mixtures of increasing methanol content. For $0.15 < x_M < 0.35$, λ_{em} increases with increasing methanol content to 525 nm and then decreases monotonically to the value it assumes in pure methanol. We confirmed that the dansyl chromophore of the model compound is "well-behaved" in water/methanol mixtures. As the methanol content in the solvent mixture increases, the emission maximum shifts monotonically to shorter wavelengths, from a value of 560 nm in pure water to 525 nm in methanol, and the fluorescence peak width at half-height increases. These values agree with data for the emission of the probe in these solvents.[36] A linear fit of the values against the changes in dielectric constants of water/MeOH mixtures confirmed the sensitivity of the probe to its environment polarity in this specific solvent mixture.

Time-Dependent Fluorescence. The fluorescence lifetime of the dansyl group is a measure of the micropolarity and the microviscosity sensed by the label. It is known that for probes kept in homogeneous solutions the dansyl emission decays exponentially with lifetimes ranging from 4 to 30 ns. Our measurements of the fluorescence decay of the model compound DaEAM in water/methanol mixtures confirmed this observation. All the curves, monitored at 550 nm, could be fit satisfactorily to a single-exponential function (eq 2). The lifetime increased monotonically with increasing methanol content from 4 ns in pure water to 17 ns in pure methanol.

$$I(t) = A \exp(-t/\tau) \quad (2)$$

The decays of dansyl groups attached to the polymer or to the gels are also monoexponential for all solvent compositions, but the plot of τ vs x_M deviates significantly from the monotonic increase observed for the model compound (Figure 4). The emission lifetime for the dansyl chromophore linked to the polymer or to the gels increases sharply in the solvent mixtures with $0.05 < x_M < 0.15$, reaching a maximum of 16.3 ns, and gradually decreases for $x_M > 0.15$ to 15 ns in pure methanol. Close examination of the curves in Figure 4 reveals the presence of a shallow minimum in τ (13.2 ns) for a solvent composition of x_M *ca.* 0.45. The lifetime values recorded in either pure water or pure methanol differ slightly from the corresponding lifetimes of the model compound. The dansyl lifetime is longer for the polymer and gel in water. In methanol the opposite situation takes place. The differences are outside the experimental error of the measurements and may reflect the impact of the polymer backbone on the polarity sensed by the probe.

Fluorescence Depolarization. A dansyl group unable to rotate freely will emit polarized light with some degree of correlation between the plane of polarized excitation and the plane of polarized emission. The anisotropy ratio r (see definition in the Experimental Section) allows us to calculate the rotational diffusion coefficient D_r from the Perrin–Weber equation (eq 3), where η is the viscosity of the solvent surrounding the probe, T is the absolute temperature, υ is the rotational volume of the dansyl group, k_B is the Boltzmann constant, and τ is the fluorescence lifetime of the probe:

$$r/r_0 = 1 + (k_B T/\upsilon\eta)\tau = 1 + 6D_r\tau \quad (3)$$

The anisotropy ratio is negligible for the model compound in solvents of low viscosity such as methanol (r = 0.0015) or water (r = 0.0007). It reaches a limiting value r_0 = 0.325 (extrapolated from highly viscous glycerol/water mixtures) in highly viscous solvents.[19] The anisotropy ratio of dansyl groups attached to PNIPAM-Da gels is rather low when the gels are in their swollen state (0.02 for gels in methanol and 0.07 for gels in water). In collapsed gels it increases dramatically to a maximum of 0.20 in a mixture containing 0.20 mol % methanol (Figure 5). But even in the collapsed gels r does not reach its limiting value, indicating that while the probe constrained in a col-

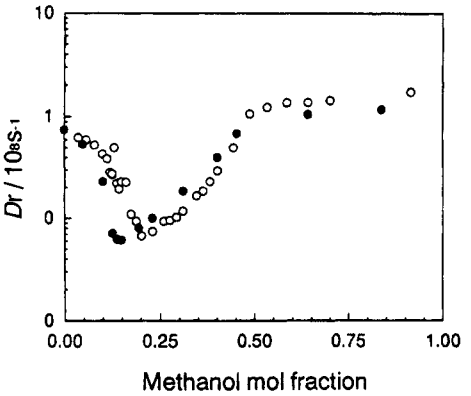

Figure 6. Changes in the rotational diffusion coefficient, D_r, of the dansyl label as a function of solvent composition in MeOH/water mixtures for PNIPAM-Da (full circles) and PNIPAM-Da gels (open circles).

lapsed gel experiences a high effective viscosity, it is still able to undergo some rotational motion. The calculated rotational diffusion coefficients of the label are 6.3×10^7 s^{-1} for swollen gels in water and 1.7×10^8 s^{-1} in methanol. In the collapsed gels D_r decreases by a factor of 10, to a minimum of 6.7×10^6 s^{-1} in a mixture of $x_M = 0.20$ (Figure 6). Note that the pattern exhibited by the curve of D_r as a function of solvent composition matches closely the swelling curve (Figure 2).

The anisotropy of the dansyl group attached to the linear polymer PNIPAM-Da was recorded also as a function of solvent composition (Figure 5). It was used to determine the rotational diffusion coefficient, D_r, of the label attached to the polymer (Figure 6). Qualitatively, the response of the chromophore to changes in solvent composition is the same as that in the gels: the anisotropy is low when the polymer exists as a freely rotating coil in solution; it is much higher in the collapsed polymer globule. However, there are significant differences between the response of the labeled polymer and the labeled gel. The most noticeable discrepancy is seen in the range of solvent compositions, for which the largest amplitude changes in r and D_r occur. The increase in r and the corresponding decrease in D_r take place for $0.05 < x_M < 0.15$ with a midpoint at $x_M = 0.10$ in the case of the labeled polymer, while for the gels the transitions occur for $0.05 < x_M < 0.20$ (midpoint of the transition: $x_M = 0.15$). At first we suspected that this difference may be related to the fact that the gels were equilibrated for 1 month in given solvent mixtures prior to spectroscopic investigation, whereas the measurements on the collapsed polymers were performed immediately after sample preparation. This concern was ruled out, by measuring over a 1-month period the anisotropy ratio r for a sample of PNIPAM-Da in a methanol/water mixture with $x_M = 0.12$ (collapsed polymer; $r = 0.189$ after 1 day, $r = 0.189$ after 2 weeks, $r = 0.187$ after 1 month). We propose that this disparity has the same origin as the observed difference between the cloud point of PNIPAM in water (31.5 °C) and the volume phase transition temperature of a methylenebis(acrylamide) crosslinked PNIPAM gel (34 °C). Otake *et al.* have shown that the introduction of crosslinking methylene bridges between poly(*N*-isopropylacrylamide) chains creates a microenvironment of increased hydrophilicity.[40] They demonstrated also that the volume phase transition of gels decreases for *N*-alkylacrylamide gels with increasingly hydrophobic amido substituents. According to the work of Mukae *et al.*[39] in a study of PNIPAM gels and related gels in water/alcohol mixed solvents, the volume phase transition shifts to lower alcohol content with increasing the number of carbon atoms in the polymer side chains. Therefore, we conclude that the differences in the dependence on solvent composition of the parameters r and D_r uncovered in this study reflect a difference in the solvent composition for which phase transition occurs either in the polymer or in the gels. It may also reveal the occurrence of preferential solvent adsorption of one solvent to the polymer or the gel. A study by EPR spectroscopy of the cononsolvency of spin-labeled poly(*N*-isopropylacrylamide)[32] has demonstrated the preferential adsorption of methanol to the collapsed polymer chains. Our results on the dansyl-labeled gels have prompted us to initiate a similar study with spin-labeled gels to examine this possibility in detail.

References and Notes

(1) Katchalsky, A. *Experientia* **1949**, *5*, 319–320.
(2) For a recent overview of the field, see: *Responsive Gels, Volume Transitions*; E. Dusek, E., Ed.; Springer-Verlag: Berlin, 1993; Vol. II, p 110. Volume Phase Transition and Related Phenomena of Polymer Gels. *Advances in Polymer Science*; Tanaka, T., Shibayama, K., Eds.; Springer-Verlag: Berlin, 1993; p 109.
(3) Hirotsu, S.; Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1987**, *87*, 1392–1395.
(4) Ilavsky, M. *Polymer* **1981**, *22*, 1687.
(5) Tanaka, T.; Hocker, L. O.; Benedek, G. B. *J. Chem. Phys.* **1959**, *59*, 5151.
(6) Peters, A.; Candau, S. J. *Macromolecules* **1988**, *21*, 2278.
(7) Mallam, S.; Horkay, F.; Hecht, A. M.; Geissler, E. *Macromolecules* **1989**, *22*, 3356.
(8) Reina, J. C.; Bansil, R.; Konak, C. *Polymer* **1990**, *31*, 1038.
(9) Tokuhiro, T.; Amiya, T.; Mamada, A.; Tanaka, T. *Macromolecules* **1991**, *24*, 2936–2943.
(10) Ohta, H.; Ando, I.; Fujishige, S.; Kubota, K. *J. Mol. Struct.* **1991**, *245*, 391.
(11) Li, Y.; Tanaka, T. *J. Chem. Phys.* **1989**, *94*, 5161.
(12) Tokida, M.; Tanaka, T. *Science* **1991**, *253*, 1121.
(13) Sato, E.; Tanaka, T. *J. Chem. Phys.* **1991**, *96*, 1695.
(14) Shibayama, K.; Tanaka, T.; Han, C. C. *J. Chem. Phys.* **1992**, *97*, 6829.
(15) Windle, J. J.; Scherrer, R. *Magn. Reson. Chem.* **1992**, *30*, 927.
(16) Hradil, J.; Konak, C.; Svec, F. *Reac. Polym.* **1989**, *11*, 147.
(17) Shea, K. J.; Stoddard, G. J.; Shavelle, D. M.; Wakui, F.; Choate, R. M. *Macromolecules* **1990**, *21*, 4497.
(18) Horie, K.; Mita, I.; Kawabata, J.; Nakahama, S.; Hirao, A.; Yamazaki, N. *Polymer J.* **1980**, *12*, 319.
(19) Hu, Y.; Horie, K.; Ushiki, H. *Macromolecules* **1992**, *25*, 6040.
(20) Hu, Y.; Horie, K.; Ushiki, H.; Tsunomori, F.; Yamashita, T. *Macromolecules* **1992**, *25*, 7324–7329.
(21) Hu, Y.; Horie, K.; Torii, T.; Ushiki, H.; Tang, X. *Polym. J.* **1993**, *25*, 123–1303. Hu, Y.; Horie, K.; Ushiki, H.; Yamashita, T.; Tsunomori, F. *Macromolecules* **1993**, *26*, 1761. Hu, Y.; Horie, K.; Ushiki, H. *Polym. J.* **1993**, *25*, 651. Hu, Y.; Horie, K.; Ushiki, H.; Tsunomori, F. *Eur. Polym. J.* **1993**, *29*, 1365.
(22) Tanaka, T. *Phys. Rev. Lett.* **1978**, *40*, 820.
(23) Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1984**, *81*, 6379.
(24) Freitas, R. F.; Cussler, E. L. *J. Chem. Phys.* **1987**, *42*, 97.
(25) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163.
(26) Amiya, T.; Hirokawa, Y.; Hirose, Y.; Li, Y.; Tanaka, T. *J. Chem. Phys.* **1987**, *86*, 2375.
(27) Hirotsu, S. *J. Phys. Soc. Jpn.* **1987**, *56*, 233.
(28) Mukae, K.; Bae, Y. H.; Okano, T.; Kim, S. W. *Polym. J.* **1990**, *22*, 250. Bae, Y.; Okano, T.; Kim, S. W. *Pharmacol. Res.* **1991**, *8*, 624.
(29) Heskins, M.; Guillet, J. E. *J. Polym. Sci.* **1957**, *25*, 43.
(30) Winnik, F. M.; Ringsdorf, H.; Venzmer, J. *Macromolecules* **1990**, *23*, 2415.
(31) Schild, H. G.; Muthukumar, M.; Tirrell, D. A. *Macromolecules* **1991**, *24*, 948.
(32) Winnik, F. M.; Ottaviani, M. F.; Bossmann, S. H.; Garcia-Garibay, M.; Turro, N. J. *Macromolecules* **1992**, *25*, 6007.

(33) Winnik, F. M.; Ottaviani, M. F.; Bossmann, S. H.; Pan, W.; Garcia-Garibay, M.; Turro, N. J. *Macromolecules* **1993**, *26*, 4577.

(34) Winnik, F. M. In *Photochemical Processes in Organized Molecular Systems*; Honda, K., Ed.; North-Holland and Elsevier Science: Amsterdam, The Netherlands, 1991; p 257.

(35) Kajima, Y.; Yagi, Y.; Inomata, H.; Saito, S. *Polym. Gels Networks* **1993**, *1*, 127.

(36) Li, Y.-H.; Chan, L. M.; Tyer, L.; Moody, R. T.; Himel, C. M.; Hercules, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 3118. Shea, K. J.; Okahata, Y.; Dougherty, T. K. *Macromolecules* **1984**, *17*, 296. Shea, K. J.; Stoddard, G. J.; Shavelle, D. M.; Wakui, F.; Choate, R. M. *Macromolecules* **1990**, *21*, 4497–4507.

(37) Gehrke, S. H.; Palasis, M.; Akhtar, M. K. *Polym. Int.* **1992**, *29*, 29.

(38) (a) Fujishige, S. *Polym. J.* **1987**, *19*, 297. (b) Hirotsu, S. *J. Phys. Soc. Jpn.* **1987**, *56*, 233.

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

(40) Otake, K.; Inomata, K.; Konno, M.; Saito, S. *Macromolecules* **1990**, *23*, 283.

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