Fluorescence Studies of the Volume Phase Transition of Poly(acrylamide) Gels with a Dansyl Group

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ABSTRACT: The changes in microenvironments during a pH-induced volume transition of poly(acrylamide) (PAAm) gels with a dansyl group as a fluorescent probe in an acetone/water (9/11) mixed solvent have been studied by the fluorescence spectrum, anisotropy, and lifetime measurements. Irrespective of the presence or absence of fluorescence probe groups labeled on the network of PAAm gels, the gels are in the collapsed state at pH = 1.0–5.0. They show a discontinuous volume phase transition (DVPT) at pH = 5.1 and remain swollen up to pH = 9.6. The fluorescence anisotropy ratio, r, also changes at pH = 5.1 for the dansyl group, but the peak shift of the fluorescence spectra of the dansyl group takes place at pH = 3.8, i.e., before the DVPT. The fluorescence decay measurements demonstrated two emitting states for the excited dansyl group depending on pH values. The microscopic changes in hydrophobicity and mobility around polymer segments are demonstrated at pH = 3.8 by the fluorescent dansyl probe, although the macroscopic DVPT occurs at pH = 5.1.

Introduction

It has been reported that poly(acrylamide) (PAAm) gels show a discontinuous volume phase transition (DVPT) with temperature, solvent composition, pH, ionic component, or a small electric field across the ionized gel.[1] The DVPT of a poly(*N*-isopropylacrylamide) (PNIPA) gel was shown by thermal analysis to be controlled by the same factors as the coil–globule transition of linear PNIPA.[2] The behavior of DVPT was interpreted by assuming that the free energy of contact between polymer segments was not a linear function of the solvent composition.[3,4] It was also interpreted in terms of the mean-field theory based on the extension of Flory's formula for the free energy of gels. In the volume change of the ionic gels ionization of the gel network plays an essential role in the DVPT,[5–8] as also indicated by results on linear PAAm.[9] The kinetics of swelling of PAAm gels was measured by light scattering,[10,11] and the diffusion coefficient of a gel network was obtained. The swelling properties and the mechanical behavior of PAAm gels were also measured by small-angle X-ray and dynamic light scattering.[7,8,12,13] The dependence of the dynamics of probe particles in poly(acrylamide) gels as a function of the cross-link content, scattering angle, and size of the probe was studied using photon correlation spectroscopy,[14] when the gel was not swollen. The dynamics of concentration fluctuations in a gel near the phase separation was described by a mode-coupling theory, and the theory was supported by the results of Tanaka.[15]

Although few papers reported the photophysical properties of a PAAm gel, many studies were devoted to the solution properties of the linear PAAm or polyelectrolyte using the fluorescence technique. The formation of the PAAm gel was monitored by means of a fluorescent probe.[16] This technique revealed that highly cross-linked PAAm gels were permeable to polar solvents and less permeable to nonpolar solvents.[17] The lower critical solution temperature (LCST) of linear PNIPA, similar to the phenomenon of DVPT of the PAAm gel, was due to the difference in interaction between polymer chains and between the polymer and water as characterized by pyrene excimer fluorescence.[18] At LCST, the conformation of linear PNIPA changed from extended coils to a compact globular state as indicated by the time-resolved fluorescence anisotropy of dansyl groups.[19]

The fluorescence technique is suitable for studying polymer network dynamics and microenvironments during the volume phase transition of PAAm gels, in particular for studying microscopically how and why DVPT occurs. The dansyl group has been widely used as a fluorescence probe to study conformational transition in proteins[20] and synthetic polymers.[21] This group has its emission intensity increase sharply and the emission maximum shift to shorter wavelengths with a change of the microenvironments to a less polar medium than water.[22,23] In the present work, changes in the microenvironment have been studied by the fluorescence spectrum, anisotropy, and lifetime measurements of dansyl labels during a pH-induced volume phase transition of PAAm gels in an acetone/water (9/11) mixed solvent. The existence of two transition points in the PAAm gel has been observed.

Experimental Section

Preparation of *N*-[2-[[[5-(Dimethylamino)-1-naphthalenyl]sulfonyl]amino]ethyl]-2-acrylamide (Dan). All reagents and solvents were obtained from commercial suppliers and were purified before use. Dan was used as a fluorescent probe monomer and was synthesized as described in ref 17.

Preparation of the Poly(acrylamide) (PAAm) Gel. The PAAm gel with the dansyl probe was prepared by copolymerization of 5.0 g of acrylamide, 0.200 g of an *N,N'*-methylenebisacrylamide cross-linker, 0.33 mL (0.335 g) of methacrylic acid, and 3.0×10^{-2} g of a monomer of Dan using 0.04 mL of a tetramethylethylenediamine (TEMED) accelerator and 40 mg of an ammonium persulfate initiator. All ingredients, with the exception of TEMED and ammonium persulfate, were dissolved in the mixed solvent of DMF/water (2/3 volume ratio). The above solution was neutralized to pH = 8.6 with 2 N NaOH in the mixed solvent of DMF/water[7] and was flushed with nitrogen, TEMED and ammonium persulfate were added, and the solution was made up to 100 mL with the mixed solvent of DMF/water (2/3 volume ratio). After stirring, the solution was flushed with nitrogen and left overnight at room temperature to polymerize. After gelation the gels were washed with water to remove residual

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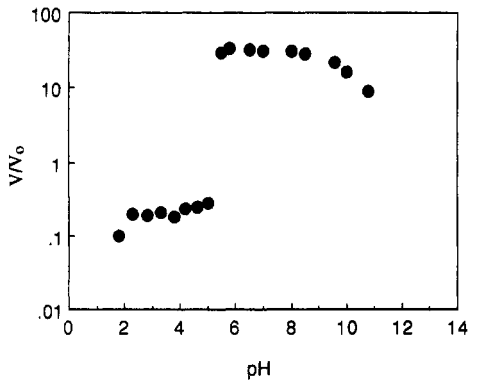

Figure 1. Degree of equilibrium swelling of a dansyl-labeled PAAm gel as a function of pH at 20 °C in acetone/water (9/11).

chemicals. The gels were cut in 1.5-cm-long samples, immersed in the mixed solvent of acetone/water (9/11) with different pH values, and then swelled or shrunk at 20 °C. In order to have the same Na^+ concentration or the same ionic strength for different swelling systems, the whole mixed solvent of acetone/water was first brought to pH = 12 and then adjusted to different pH values with hydrochloric acid. After reaching swelling equilibrium, the volume and photophysical properties of the PAAm gels were measured.

Preparation of Linear Poly(acrylamide) (PAAm). A linear PAAm with the fluorescent probe was prepared using the same preparation procedure as for the PAAm gel except for omitting the cross-linking reagent. After polymerization, the polymer was precipitated with methanol. Subsequent purification involved dissolving the polymer in DMF, reprecipitating with methanol, and drying under vacuum.

Measurements of Photophysical Properties. UV absorption spectra of all of the samples were recorded with a Jasco 660 UV/vis spectrophotometer. The excitation spectra, emission spectra, and fluorescence anisotropy ratios were measured with a Hitachi 650-40 fluorescence spectrophotometer. The fluorescence decays were obtained by a photon-counting apparatus (Horiba NAES-1100). For measurements of spectra of dansyl probe labeled samples, the excitation wavelength was set at 345 nm. Slit widths ranging from 2 to 7 nm were chosen, depending on the pH value. The values of the steady-state fluorescence anisotropy ratio were averaged in the region of the peak wavelength ±20 nm.

The PAAm gels which were swollen in the mixed solvent of acetone/water (9/11) at a given pH were cut to a suitable size and placed into a quartz cell, and the original mixed solvent of acetone/water (9/11) with the same pH was added to equilibrate with the gel. All spectra were obtained in quartz cells in content with the atmosphere. The linear PAAm was dissolved in the mixed solvent acetone/water (9/11) at a definite pH. The mixed solvents were adjusted to the same Na^+ concentration as the gels. The concentration of the polymer was 2.74×10^{-2} g/mL. All spectra were measured in a quartz cell under the same conditions as the gels.

Results

Synthesis and Characterization of the PAAm Gel and Linear PAAm. According to the experiments of Tanaka,[5] the PAAm gels having no ionic groups were placed into a basic solution in water to hydrolyze a portion of the acrylamide to acrylic acid residues. Ilavsky[7] directly used sodium methacrylate to prepare PAAm gels with ionic groups. In our work, the methacrylic acid was neutralized to pH 8.6 before polymerization. The PAAm gel and linear PAAm were copolymerized by the monomers to random copolymers with sodium methacrylate.[24] The gels which were prepared both from methacrylic acid and sodium methacrylate exhibit identical properties and DVPT in a forthcoming publication.

Figure 1 shows the relationship of pH to the degree of equilibrium swelling of gels [$V/V_0 = (D/D_0)^3$]. V and V_0 are the volumes and D and D_0 are the sample diameters after equilibrium swelling and at the time of preparation of the PAAm gels, respectively. Irrespective of the presence or absence of dansyl labels on the gels, the gel is in the collapsed state in the range from pH = 1.0 to 5.0. At pH = 5.1 the gel shows a discontinuous volume phase transition (DVPT) and remains swollen up to pH = 9.6. After swelling, the PAAm gels are transparent solids at all pH. After pH = 10.8, acrylamide residues are hydrolyzed and the bonds of dansyl groups to the PAAm main chain are also destroyed so that the solvent becomes yellow.

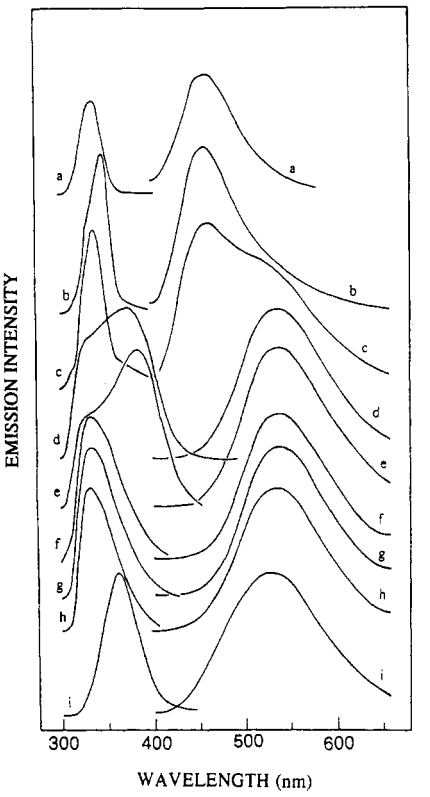

Figure 2. Typical excitation and emission spectra of a dansyl probe attached to the PAAm gels after equilibrium swelling at pH = 1.8 (a), 2.8 (b), 3.3 (c), 4.2 (d), 5.0 (e), 6.5 (f), 7.0 (g), 9.6 (h), and 10.0 (i), respectively. Fluorescence spectra are excited at 345 nm, and excitation spectra are monitored at the peak wavelength of the fluorescence spectra.

Steady-State Photophysical Properties of Dansyl Groups Attached to the PAAm Gel and Linear PAAm. Both PAAm gel and linear PAAm exhibit an absorption maximum at 333 nm in the mixed solvent for different pH. In order to avoid absorption by acetone in the mixed solvent below 325 nm, 345 nm is chosen as an excitation wavelength both for the PAAm gel and for the linear PAAm. Because the gels in the mixed solvent with different pH values have different sizes, the absolute fluorescence intensities for the gels with different pH values are not significant. Typical excitation and emission spectra of the dansyl group attached to the network of the PAAm gel are shown in Figure 2 for various pH values. The fluorescence spectra exhibit two peaks at 460 and 540 nm. The changes in the fluorescence peak wavelength and the fluorescence intensity ratio of dansyl groups in the PAAm gel and linear PAAm are plotted against pH in Figures 3 and 4. The shift of the fluorescence maxima, λ_{EM}, takes place at pH = 3.8 before the DVPT. The fluorescence intensity at 540 nm (I_{540}) increases gradually from pH = 2.8 to 4.6 and remains a constant above pH = 5.0. The fluorescence intensity at 460 nm (I_{460}) decreases gradually and almost disappears above pH = 5.0.

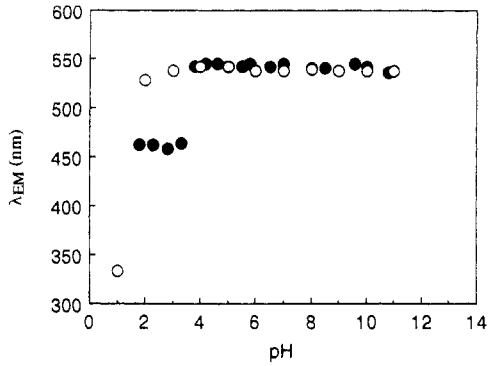

Figure 3. pH dependence of the fluorescence peak wavelength of the dansyl probes attached to the PAAm gel (●) and linear PAAm (○).

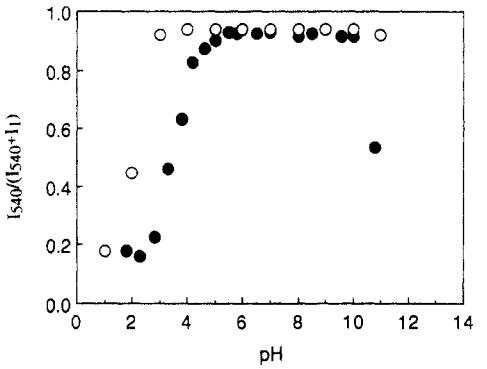

Figure 4. pH dependence of the relative intensity of the 540-nm fluorescence for the dansyl-labeled PAAm gel (●) and linear PAAm (○). I_1 is the intensity at 336 nm for linear PAAm below pH = 2.0, and I_1 is the intensity at 460 nm for the PAAm gel at all pH values and for linear PAAm above pH = 2.0.

According to the results of Strauss and Vesnaver,[21] there exist two isosbestic points at 268 and 304 nm in the absorption spectra of dansyl groups in copolymers of maleic anhydride and vinyl alkyl ethers, and the absorption between 268 and 304 nm belongs to the protonated and that above 304 nm to the unprotonated *N*-dimethylamino moiety of the dansyl group. The acid–base equilibrium in a water solution with 0.5 N NaCl was characterized by $pK_d = 3.9$. The emission spectra are characterized by two bands with maxima at 336 and 580 nm which correspond to fluorescence from the excited states of the protonated and unprotonated forms, respectively, with excitation at 268 nm. In the present work this absorption isosbestic point of the dansyl group due to the protonation of the dimethylamino moiety was not observed owing to acetone absorption, and the excitation wavelength was set at 345 nm. The present results showing emission at 460 or 540 nm for the gels are different from the results of Strauss and Vesnaver. Although the fluorescence of dansyl groups in linear PAAm can be found at 336 nm in the region of pH = 1.0–2.0 due to the protonated dansyl groups attached to the linear PAAm, the fluorescence in the PAAm gel was found only at 460 nm at pH = 1.8, due to the special microenvironment in the PAAm gel different from the linear PAAm. It is well-known that[22,23,25] there exist two emitting states for excited dansyl groups, which respond differently to the medium polarity and viscosity. This phenomenon is related to the formation of a twisted intramolecular charge transfer (TICT).[26–29] For PAAm gels, the TICT occurs at pH = 3.8 before DVPT.

Fluorescence Transient Decays of Dansyl Groups Attached to PAAm Gel and Linear PAAm. Just as the steady-state data show two emitting stages of dansyl groups on side chains of the gel network, two emitting states are also indicated by the fluorescence transient decays shown in Table I. The monomer decay curves typically shown in Figure 5 could be satisfactorily fitted with a double exponential for PAAm gels and linear PAAm.

$$I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) \quad (1)$$

The apparent fractions of the fluorescence of the shorter lifetime component, $Q_1 = A_1\tau_1/\sum A_i\tau_i$, are also given in Table I. For PAAm gels, at pH = 1.8–3.3, the component with the shorter decay time is smaller; above pH = 5.0 all of the emitting states of the dansyl group correspond to the component with the shorter decay time, and when the gels are in the collapsed state in the range of pH = 3.3–5.0, there exist two emitting states. This result is identical to the result of the relative fluorescence intensity ratio in steady-state fluorescence spectra. The longer decay time corresponds to the coplanar excited state, and the shorter decay time corresponds to the TICT excited state. The fluorescence peak at 336 nm in linear PAAm with a short lifetime of 2.7 ns at pH = 1.0 would be due to the protonated dansyl group. At pH = 10.8, the dansyl groups were destroyed and the steady-state fluorescence spectra and fluorescence transient decay behave abnormally with a 500-nm emission and a much shorter decay time.

Figure 6 shows the dependence of the fluorescence anisotropy ratios, r, on pH, which are about 0.06 for pH = 1.8–5.0 in PAAm gels and becomes about 0.03 at pH = 5.5, which is the same as for linear PAAm at all pH values. The pH dependence of the fluorescence anisotropy ratio of dansyl groups bonded to the PAAm gel coincides with that of the gel volume.

Based on the Perin–Weber equation

$$r_0/r = 1 + (k_B T/v\eta)\tau = 1 + 6D_r\tau \quad (2)$$

where D_r is the rotational diffusion coefficient, τ is the lifetime of the fluorescence, η is the viscosity of the solvent, v is the rotational volume of the dansyl group, and r_0 is the limiting value of r in a medium where the Brownian motion is frozen. The value of r_0 was determined to be 0.325 by extrapolating the values of r to infinite viscosity for the probe monomer with the dansyl groups, Dan, in glycerol/water mixtures.

The rotational diffusion coefficient, D_r, calculated from the values of r and τ by using eq 2 is given in Figure 7. The values of D_r at below pH = 3.3 correspond to D_r of the coplanar excited state of dansyl groups in the gel with the longer decay time τ_2, the values of D_r at above pH = 5.0 correspond to D_r of the TICT excited state of dansyl groups in the gel with the shorter decay time τ_1, and the values of D_r in the region from pH = 3.8 to 4.6 are given for both the TICT excited state and the coplanar excited state of dansyl groups in the gel. In conformity with the results of steady-state fluorescence, the rotational diffusion coefficient of dansyl groups attached to the PAAm gel exhibits three regions, that is, pH below 3.8, above 5.0, and between 3.8 and 5.0, while the value of the rotational diffusion coefficient of dansyl groups attached to linear PAAm exhibits no marked change over the whole pH range of the present study with the value near to that in the PAAm gel above pH = 5.0.

Discussion

The change in the emitting states of dansyl groups could be ascribed to the formation of the TICT state,[26–29] induced by the changes in micropolarity[22,23,25] or free volume[27–29] surrounding the probe. Owing to the formation of TICT, the energy level of the TICT emitting state becomes lower than that of the usual coplanar emitting state, the peak

Table I
Fluorescence Decay Parameters for Dansyl Groups Attached to the PAAm Gel and Linear PAAm

	PAAm gel					linear PAAm				
pH	A_1	τ_1 (ns)	A_2	τ_2 (ns)	Q_1[a]	A_1	τ_1 (ns)	A_2	τ_2 (ns)	Q_1[a]
1.0						0.1340	2.70	0.0383	8.48	0.527
1.8	0.0731	4.61	0.0634	12.4	0.300					
2.3	0.0596	5.60	0.0736	13.7	0.248					
2.8	0.0497	5.61	0.0749	13.6	0.215					
3.0						0.0976	6.95	0.0187	15.0	0.707
3.3	0.0565	4.72	0.0705	13.2	0.223					
3.8	0.0877	6.31	0.0321	15.7	0.523					
4.2	0.0999	6.04	0.0283	14.3	0.598					
4.6	0.1100	6.24	0.0179	16.5	0.699					
5.0	0.1010	6.04	0.0156	15.3	0.719					
5.5	0.1040	6.26	0.0133	14.5	0.771					
5.8	0.0966	5.98	0.0138	14.1	0.748					
6.5	0.1270	6.44	0.0188	15.1	0.742					
7.0	0.1210	6.22	0.0151	15.8	0.759	0.0902	7.02	0.0298	14.4	0.596
8.0	0.1680	6.44	0.0189	15.5	0.787					
8.5	0.0992	6.29	0.0133	15.2	0.755					
9.6	0.1720	6.13	0.0173	14.6	0.807					
10.0	0.1460	6.10	0.0178	14.1	0.780					
10.8	0.1530	2.22	0.0418	6.72	0.547					
11.0						0.0850	6.92	0.0271	13.5	0.616

[a] $Q_1 = A_1\tau_1/(A_1\tau_1 + A_2\tau_2)$.

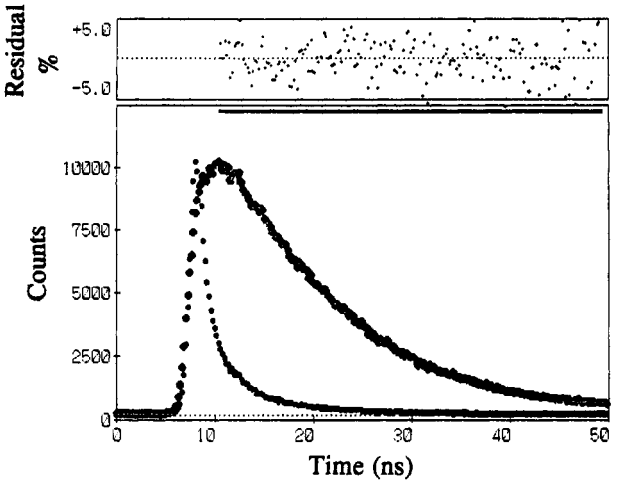

Figure 5. Fluorescence decay and a residual for curve fitting by eq 1 of the dansyl probe attached to the PAAm gel at pH = 3.8 (for lifetime, see Table I).

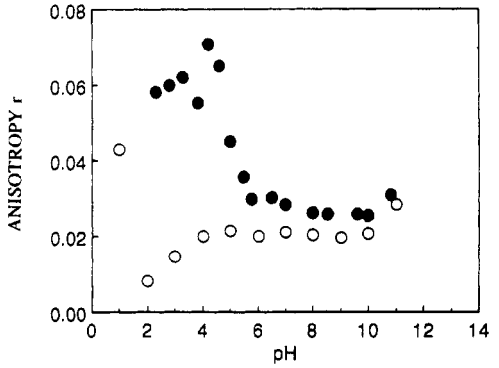

Figure 6. Plots of the fluorescence anisotropy ratio, r, of the dansyl probes attached to the PAAm gel (●), and side chains of linear PAAm (○), against pH. Values of r are the average ones in the region of peak wavelength ±20 nm. At pH = 1.8 for the PAAm gel, r = 0.135.

position of fluorescence wavelength shifts to the red side, and the fluorescence quantum yield of the dansyl group decreases. The reduced mobility of the dansyl group bound to the shrunk polymer network and the hydrophobicity of the microenvironment surrounding the dansyl group at low pH restrict the electron-transfer process

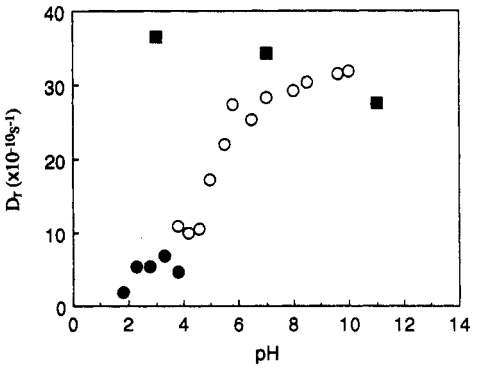

Figure 7. Changes in the rotational diffusion coefficient, D_r, of dansyl probes attached to the PAAm gel (●, ○), and linear PAAm (■), against pH by using the Perin–Weber equation with the values of τ_1 (○, ■) and τ_2 (●) and r_0 = 0.325.

between these two states within the lifetime of the excited state. The different microenvironments between linear PAAm and the PAAm gel are reflected by the dansyl fluorescent probe, since the networks of the PAAm gel are characterized by a high density of monomer residues, while dansyl groups attached to linear PAAm are in much more intimate contact with solvent molecules.

In our results, there exist two transition points in the PAAm gel system. One occurs at pH = 3.8 which is a microscopic transition point (MITP) for the change in the peak shift of fluorescence spectra in Figure 3, for the change in the relative fluorescence intensity between two components in Figure 4, and for the change in the apparent fraction Q_1 in Table I. This MITP is different from the DVPT of the PAAm gel. The other occurs at pH = 5.0 as a macroscopic transition point (MATP) that is similar to the DVPT of the PAAm gel in Figure 1 and identical to the transition point of the fluorescence anisotropy ratio in Figure 6. The changes in the rotational diffusion coefficient D_r are observed at both pH = 3.8 and pH = 5.0 in Figure 7 for the dansyl group attached to the PAAm gels.

Below the MITP of pH = 3.8, because the ionic strength or the solvent polarity is weaker, the dansyl groups attached to the PAAm gels seem to be very tightly entrapped as in supercoiled poly(methacrylic acid),[30] forming a highly hydrophobic microenvironment. Because

of the high rigidity of the network and the highly hydrophobic microenvironment, the transition between different emitting states of the probe cannot be brought about by most of the dansyl group. The mobility of the dansyl moiety is highly restricted. In the region between the MITP and MATP at pH = 3.8–5.0, because the microenvironment is less hydrophobic, the transition to the TICT state can be realized. But in this region of pH, the mobility of the naphthyl group is still restricted, because of the compactness of the network. Above the region of MATP at pH = 5.0, where the gel swells due to the repulsion between the ionic charge, the microenvironment of dansyl groups attached to the PAAm gel becomes hydrophilic and the TICT is formed by all of the emitting dansyl groups in the gel.

Conclusion

Poly(acrylamide) (PAAm) gel with methacrylic acid residues carrying fluorescent dansyl groups was synthesized. Changes in the microenvironment, interaction between network chains, and interaction of network chains with solvent during pH-induced discontinuous volume phase transition (DVPT) of the PAAm gel are demonstrated by the fluorescence properties of dansyl groups attached to the PAAm gel. Two transition points of the PAAm gel are reflected by fluorescence, one at pH = 3.8, which is a microscopic transition point (MITP), and the other at pH = 5.0, a macroscopic transition point (MATP) coinciding with the DVPT. At a pH below MITP, the formation of a twisted intramolecular charge transfer (TICT) of dansyl groups attached to the PAAm gel is not realized owing to the highly hydrophobic microenvironments and the compactness of PAAm networks. In the region between MITP and MATP, the TICT can be realized for most of the dansyl groups due to a less hydrophobic microenvironment, but the PAAm networks are still more rigid. At a pH above MATP, the TICT state is observed for all of the dansyl groups in the gel, and the PAAm network clusters disintegrate due to the repulsion of ionized carboxyls.

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Registry No. Dan/acrylamide/*N,N'*-methylenebisacrylamide/methacrylic acid (copolymer), 141848-40-0; Dan/acrylamide/methacrylic acid (copolymer), 141879-13-2.