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SHORT COMMUNICATIONS

Comparison of Steady-State and Dynamic Fluorescence Anisotropy of Dansyl Probe in the Volume Phase Transition of Poly(acrylamide) Gel

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In our previous work,[1] the micro-environment inside a poly(acrylamide) (PAAm) gel and the dynamics of the PAAm network were studied by measuring steady-state fluorescence spectra, anisotropy and lifetimes of the dansyl probe attached to the gel during the volume phase transition (VPT) of the PAAm gel induced by pH change. The results indicated that the change in the rotational diffusion motion of the dansyl probe attached to the PAAm networks caused by the change in micro-environment around the probe could be monitored by the steady-state fluorescence anisotropy. The gel showed a VPT at pH = 5.1. Two transition points were reflected by fluorescence spectroscopy, one at pH = 3.8 being a microscopic transition point corresponding to the shift of fluorescence peak wavelength, the other at pH = 5.0 being a macroscopic transition point corresponding to the change in rotational diffusion coefficient and coinciding with the VPT.

The dynamic fluorescence anisotropy technique has been widely used for studying segmental mobility and conformational changes in polyelectrolyte,[2] linear PAAm,[3] poly(*N*-isopropylacrylamide) (PNIPA),[4] and polystyrene.[5] In the present work, the results of dynamic fluorescence anisotropy will be compared to the previous results of steady-state fluorescence anisotropy for the same samples and same measurement conditions.

The PAAm gel consists of 93.0 mol% acrylamide, 5.3 mol% sodium methacrylate, 1.7 mol% *N*,*N*′-methylene-bis-acrylamide, and 0.11 mol% *N*-[2-[5-dimethylamino-1-naphthalenesulfonylamino]ethyl]acrylamide, and is swollen or collapsed in an acetone–water (9 : 11) mixed solvent by the pH control with sodium hydroxide and hydrochloric acid. The linear PAAm copolymer consists of the same components of the monomers but without *N*,*N*′-methyl-bisacrylamide.

The steady-state rotational diffusion coefficient, D_r, was calculated in our previous work[1] by using Perrin–Weber eq 1.

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} = r_0/(1 + 6D_r\tau) \qquad (1)$$

where $I_{\parallel}$ and $I_{\perp}$ are fluorescence intensities measured parallel and perpendicular to the vertically polarized excitation, respectively, r_0

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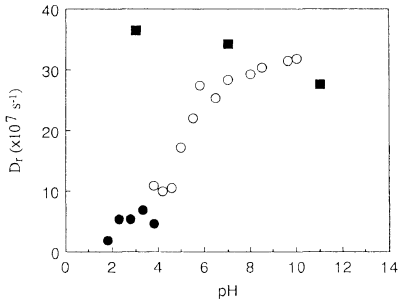

Figure 1. Changes in the rotational diffusion coefficient, D_r, of dansyl probes attached to the PAAm gel (● ○), and linear PAAm (■), against pH by using the Perrin–Weber equation with a major component of the lifetimes and $r_o = 0.325$.

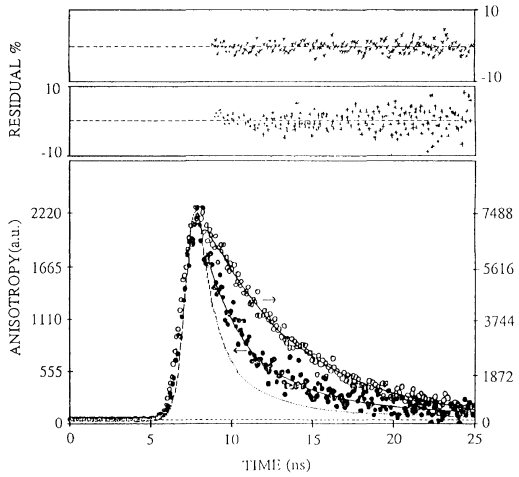

Figure 2. Fluorescence anisotropy decay curve of the dansyl probe attached to the PAAm gel (○) and its residual (×) at pH = 3.8 and the linear PAAm (●) and its residual (+) at pH = 2.0 fitted with equation 2. Dotted line shows pulse lamp profile.

is the limiting value for steady-state fluorescence anisotropy, r, in the medium where no rotational diffusion occurs or the Brownian motion is frozen, and τ is the lifetime of the probe. The obtained D_r values* are reproduced in Figure 1 against pH. The D_r values of the dansyl probe in the gel change from $7 \times 10^7\ s^{-1}$ for pH < 3.8 to more than $2.0 \times 10^8\ s^{-1}$ for pH > 5.0. The transition point of the D_r corresponds to the macroscopic VPT.

In the time-resolved approach, the fluorescence anisotropy decays, $r(t)$, were obtained by using eq 2 from the transient fluorescence decays at 550 nm, $I_{\parallel}(t)$ and $I_{\perp}(t)$, measured parallel and perpendicular to the vertically polarized excitation at 345 nm, respectively, with a Horiba MAES-1100 photoncounting apparatus. The obtained fluorescence anisotropy decays, which are typically shown in Figure 2, were analyzed by the Marquardt method[6] of convoluting twice the system response functions and varying the parameters of the fitting functions until the best least-squares agreement with experiments was obtained with a double-exponential function of eq 2.

$$r(t) = \frac{I_{\parallel}(t) - I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)} = A_1 \exp(-t/\tau_1^R) + A_2 \exp(-t/\tau_2^R) \quad (2)$$

where τ_1^R and τ_2^R are rotational diffusion lifetimes and A_1 and A_2 are their pre exponential factors.

By adopting the sphere-like model[4] of the dansyl probe attached to the polymer side chain, the rotational diffusion coefficient around lever side chain axis, D_3, and the motions perpendicular to lever axis, D_1 or D_2 ($= D_1$) can be obtained by using eq 3.

$$(\tau_1^R)^{-1} = 6D_1\ ; \quad (\tau_2^R)^{-1} = 2D_1 + 4D_3 \quad (3)$$

The obtained values for τ_1^R, τ_2^R, D_3, and D_1 are listed in Table I. According to the model described in Ref 4, the dansyl probe with the sphere radius, a, is attached to PAAm network by means of a lever length, $d+p$, where d corresponds to the distance between the center of the photophysical active part of the probe and the joint of the probe to the PAAm network and p is the dynamic persistence length. The diffusion coefficients can be related to the parameters $p+d$ and a by using the Stokes–Einstein type eq 4.

* The unit for D_r in the ordinate of Figure 6 in ref 1 should be corrected to $10^7\ s^{-1}$.

Table I. Fluorescence anisotropy decay parameters for dansyl probe attached to the PAAm gel and linear PAAm

pH	τ_1^R (ns)/A_1	τ_2^R (ns)/A_2	D_1 ($10^7 s^{-1}$)	D_3 ($10^7 s^{-1}$)	$(d+p)/a$
2.0[a]	4.5/0.034	0.42/0.29	3.7	58	4.6
2.3	12.0/0.11	2.5/0.22	1.4	9.2	3.0
3.8	11.8/0.12	2.1/0.21	1.4	11	3.3
7.0	6.0/0.045	0.73/0.28	2.8	33	4.0

[a] Linear PAAm.

$$D_1 = D_2 = \frac{kT}{(6\pi\eta a)(d+p)^2}; \quad D_3 = kT/8\pi\eta a^3 \tag{4}$$

The change in D_3 which reflects the rotational motion around the connection lever axis is caused by the change in micro-environment around the probe. The pH dependence of the rotational diffusion coefficients of the probe by dynamic measurements, D_3, is in good agreement with that of the D_r of the probe by steady-state measurement in the same condition. For example, in the pH range below 3.8 corresponding to the microscopic transition point, the D_r from the state-steady anisotropy in Figure 1 is about $7.0 \times 10^7 s^{-1}$ which is similar to the $D_3 = 9.2 \times 10^7 s^{-1}$ from the dynamic anisotropy at pH = 2.3. At pH 3.8 the $D_3 = 1.1 \times 10^8 s^{-1}$ from the dynamic anisotropy give the same value ($1.1 \times 10^8 s^{-1}$) as the D_r from state-steady anisotropy in Figure 1. At pH 7.0 for swollen state of the gel, the D_r from state-steady anisotropy in Figure 1 is about $3.0 \times 10^8 s^{-1}$ which is identical with the $D_3 = 3.2 \times 10^8 s^{-1}$ from the dynamic anisotropy at the same pH value. Thus, by using this sphere-like model,[4] the dynamic fluorescence anisotropy confirms the results of our previous work[1] on the change in micro-environment of PAAm gel during pH-induced volume phase transition. The rotational diffusion coefficient around the connection lever axis, D_r in Figure 1 or D_3 in Table I, increases about 4 times from the collapsed state to the swollen state of the gel, showing the increase in local mobility of the probe attached to the PAAm polymer.

The values of D_1 are much smaller than those of D_3, and the extent of change in D_1 of the probe in the gel with the increase in pH is less than that in D_3. This would be due to the fact that the motion of the probe in the gel perpendicular to lever axis are restricted by the connecting chain between the dansyl photophysical active center and PAAm network. The ratio of $(d+p)/a$ can be obtained from equation 4 with a constant d, which is listed in Table I. The more flexible PAAm segment in the swollen state of the gel at pH = 7.0 give larger values of D_1 and $(d+p)/a$ than those for the gels in the collapsed state. The slight increase in $(d+p)/a$ in the swollen state suggests that a decrease in p in the swollen PAAm gel due to the increase in flexibility of PAAm segment is canceled by the apparent decrease in a due to the removal of constraint against the motion of the probe.

In conclusion, the measurements of dynamic fluorescence anisotropy of PAAm gel with dansyl probe revealed the changes in rotational diffusion coefficients both around the lever axis, D_3, and perpendicular to the lever axis, D_1, during the pH-induced volume phase transition of the gel. The D_3 reflects directly the change in micro-environment around the probe, while D_1 is influenced by segmental mobility of PAAm chain.

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