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Dynamic fluorescence quenching precedent to thermally-induced phase separation of poly(ethoxyethyl vinyl ether) aqueous solution

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Summary

We focused on the stage preceding the thermally-induced phase separation of aqueous solution of poly(ethoxyethyl vinyl ether) (PEVE). Previously, we observed an interesting dynamic quenching just below the phase separation temperature. The dynamic fluorescence quenching disappeared by addition of a surfactant. In systems without the phase separation of both the hexane solution of PEVE and the PEVE bulk, the fluorescence lifetime decreased monotonically with the increase of temperature. These results indicated that the marked decrease is due to the dynamic quenching by the collision between the fluorescent probe and the PEVE segment induced by the thermal fluctuation precedent to the phase separation.

Introduction

The discontinuous changes in physical properties of polymers at a certain condition have attracted a great deal of attention from both theoretical and experimental viewpoints(1-12, 14-17). Although in general, polymers become more soluble with the increase in temperature, several polymer/solvent systems cause so-called LCST-type phase separation at a certain temperature and the polymers become insoluble above it. The dynamics of the phase separation has been studied extensively(1,3,5,8,17), but the molecular-level details of the phase separation behavior remain to be solved.

The experimental techniques using a fluorescent probe are powerful to examine the microscopic structure and dynamics. E Winnik examined the thermoreversible phase separation of aqueous solution of poly-(*N*-isopropylacrylamide) (PNIPAM) by the energy transfer technique(8). Meewes et al. used the fluorescence depolarization technique as well as the light scattering technique for the phase separation of high molecular weight PNIPAM aqueous solution(6,7). Hu et al. studied the volume phase transition in poly(acrylamide) gels using the fluorescent technique and discussed the interaction between the polymer network and solvent molecules as well as the dynamic fluctuation at the discontinuous transition(3,19,20).

An aqueous solution of poly(vinyl ether) derivatives induces the LCST-type phase separation and its behavior has been examined(9,10). Previously, we examined scattering light intensity, fluorescence intensity, fluorescence lifetime, and fluorescence depolarization during the course of phase separation of poly(ethoxyethyl vinyl ether)

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(PEVE) aqueous solution(18). Using a unique terminator, we obtained PEVE with the anthryl-group labeled in the middle of the main chain. We determined the phase separation temperature, T_{sp}, to be 20 °C by the light scattering technique. The fluorescence intensity and lifetime changed quantitatively similarly against temperature and markedly decreased between 16 °C and 20 °C, just below T_{sp}. On the other hand, the relaxation time of the local motion for PEVE estimated from the fluorescence anisotropy increased at T_{sp}. We proposed that in the range from 16 °C to 20 °C, the amplitude of thermal fluctuation of segment density becomes large precedent to the phase separation. At 20 °C, intra- and intermolecular aggregation due to the phase separation suppressed the chain mobility and the dynamic quenching disappeared. However, we did not have any other experimental results to support our proposition. Therefore, further investigation was needed especially on the quenching precedent to the phase separation.

In the present study, we focused on the fluorescence quenching precedent to the phase separation of PEVE in an aqueous solution and further examined it by comparison with other systems, where the phase separation does not occur. The purpose of this study is to ensure the correlation between the fluorescence quenching and the phase separation and to elucidate the mechanism of the quenching.

Experimental

Sample preparation.

The poly(ethoxyethyl vinyl ether) (PEVE) sample was synthesized by living cationic polymerization. Details of the material preparation and polymerization were as described elsewhere(10,13). Figure 1 shows the molecular structure of the labeled PEVE sample. PEVE used for measurements had a M_w of ca. 2 x 10^4 by GPC reduced as polystyrene. Water (Dojin nonfluorescence) and hexane (Dojin Spectrosol) were used as solvents without further purification. Sodium dodecyl sulfate (SDS) (Nacalai Tesque) was used as a surfactant for PEVE aqueous solution. Hexane solution of PEVE, which does not induce the phase separation, was prepared and the absorbance of the solution at 411 nm was ca. 0.1. PEVE bulk, which melts at room temperature, was applied to a quartz substrate. Each sample was put into a quartz cell and was degassed.

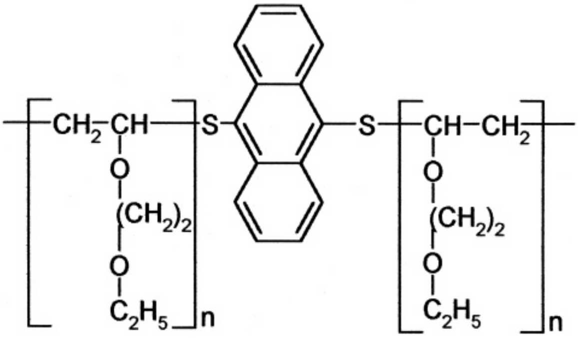

Figure 1. Molecular structure of poly(ethoxyethyl vinyl ether) (PEVE) labeled with anthryl-group in the middle of the main chain.

Fluorescence lifetime measurement.

We carried out time-resolved fluorescence measurement and estimated the fluorescence lifetime for aqueous solutions of PEVE both with and without SDS, for the hexane solution of PEVE and for PEVE bulk. The time-resolved fluorescence intensity was measured by the single-photon counting system, which was the same as that used in the previous study(18). The excitation light was polarized vertically, and the parallel and the perpendicular fluorescence components to the plane of polarization were measured. The parallel component, $I_{//}(t)$, and the perpendicular component, $I_{\perp}(t)$, were measured alternately to avoid data distortions due to the time drift.

The total fluorescence intensity is equivalent to $I(t)$ defined as eq 1

$$I(t) = I_{//}(t) + 2GI_{\perp}(t) \tag{1}$$

where G is the compensating factor. To the experimental $I(t)$ data we fitted the equation in which eq 2 was convoluted with the instrumental function by nonlinear-least-squares method.

$$I(t) = x_1 \exp(-t/\tau_1) + x_2 \exp(-t/\tau_2) + (1-x_1-x_2)\exp(-t/\tau_3) \tag{2}$$

In general, the fluorescence decay is represented by the sum of multiple exponential terms, but in this study, the observed fluorescence decays could be simulated by the triple-exponential function very well. We calculated the mean fluorescence lifetime $<\tau>$ by eq 3 with the parameters obtained from the fitting.

$$<\tau> = x_1 \tau_1 + x_2 \tau_2 + (1-x_1-x_2)\tau_3 \tag{3}$$

Results and discussion

Aqueous solution of PEVE.

Figure 2 shows the plot of (a) fluorescence intensity and lifetime and (b) the relaxation time T_m for the local motion of PEVE from the fluorescence anisotropy measurement against temperature, which was obtained previously(18). Marked

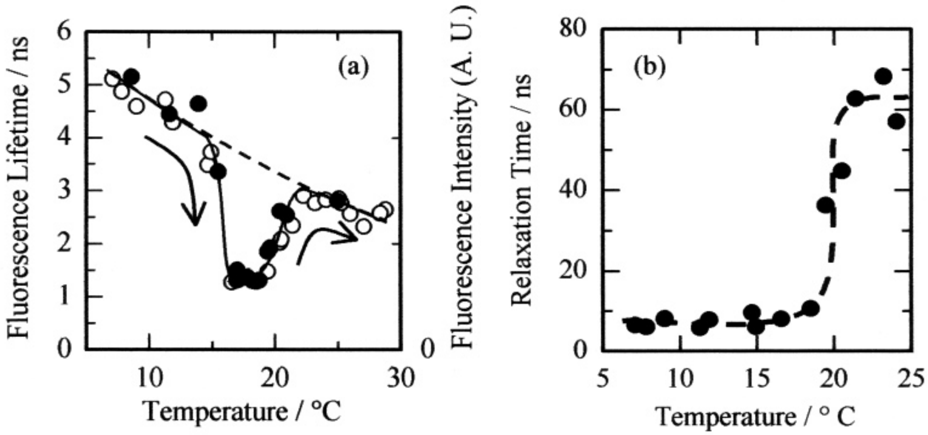

Figure 2. (a) Plots of fluorescence intensity and fluorescence lifetime $<\tau>$ against temperature in PEVE aqueous solution (0.02 wt%); fluorescence intensity (●) and fluorescence lifetime (○). The broken line is described on the assumption that the dynamic quenching does not exist. (b) Plots of the relaxation time T_m for the local motion of PEVE against temperature.

decreases of both the fluorescence intensity and <τ>, in particular, of the fraction of the longest component of <τ> were observed between ca. 16 °C and 20 °C (Figure 2(a)), and an effective quenching was proposed to occur in this region. On the other hand, the fact that the chain mobility in Figure 2(b) did not change in that temperature range means that the phase separation which results in the suppression of the chain mobility by polymer aggregation does not occur in this region. Then, we propose that the dynamic fluorescence quenching effectively occurs by collision between the fluorescent probe and the PEVE segments at the stage preceding the phase separation.

Halary et al. studied the ternary blend film of poly(vinylmethylether) (PVME), polystyrene, and labeled polystyrene(1), and found that PVME quenches fluorescence emission of labeled anthracene. Although they considered that the mechanism of the fluorescence quenching is static with the lifetime remaining constant, we found that the fluorescence intensity changed corresponding to the change of the fluorescence lifetime (Figure 2). Therefore, we believe that the decrease in the fluorescence intensity is due to the so-called dynamic quenching, in which the fluorescence intensity becomes proportional to the fluorescence lifetime.

Other systems without phase separation.

Figure 3 shows that in hexane solvent, the fluorescence lifetime of PEVE decreases monotonically and gradually with the increase of temperature. As was expected, no fluorescence quenching was observed when no phase separation took place. This result supports the proposition that the dynamic quenching takes place at the stage preceding the phase separation.

Figure 3 also shows that the fluorescence lifetime in the PEVE bulk decreases monotonically and gradually with the increase of temperature without marked quenching. Although the gradual decrease is similar to that in the hexane solution, it is noteworthy that the value of fluorescence lifetime is sufficiently lower than that of

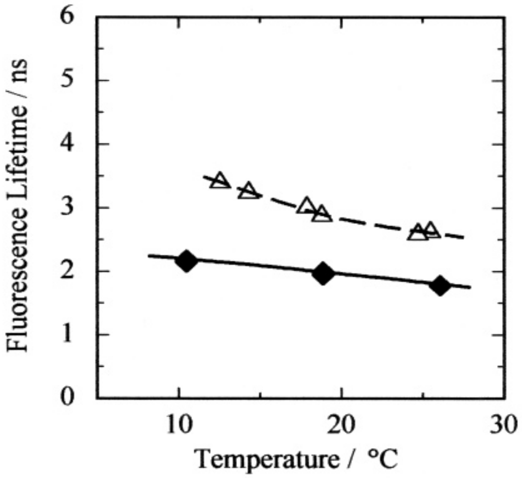

Figure 3. Fluorescence lifetime <τ> of PEVE in hexane (0.02 wt%) (△) and in PEVE bulk (◆), where no phase separation occurred.

hexane solution and near the value of dynamically-quenched lifetime in Figure 2, where the fraction of the longest component was small. In the bulk, the PEVE segment, which is considered as a quencher, exists at a high concentration (ca. 10 M) and may have mobility to some extent, and so the dynamic quenching takes place at every temperature measured. That is, the frequent collision between the fluorescence probe and PEVE segment results in the quenching of the fluorescence in PEVE bulk like the stage preceding the phase separation.

Effect of surfactant.

Meewes et al. studied surfactant effects on PNIPAM aqueous solution, which has LCST, by light scattering(7). They showed that intermolecular aggregation was completely inhibited by addition of SDS up to 250 mg L^{-1}. Moreover, at an SDS concentration of 300 mg L^{-1}, intramolecular swelling was induced by the surfactant both above and below the LCST. That is, above the LCST, the radius of a particle markedly decreased by addition of SDS up to 250 mg L^{-1} due to the intermolecular dissociation and increased by successive addition of SDS due to the intramolecular swelling. Below the LCST, where an intermolecular dissociation was not involved, only the intramolecular coil swelling was induced by SDS. They considered that the surfactant binds cooperatively to the polymer chain and at the critical concentration of 300 mg L^{-1}, the repulsive interactions between the firmly adsorbed amphiphiles becomes enough to influence the polymer conformation. They proposed that the critical concentration is close to the concentration that the surfactant is adsorbed cooperatively in the form of micelles. Thus, SDS was expected to prevent both the phase separation and the preceding fluctuation of the segment density in PEVE aqueous solution as well. Then we measured the fluorescence lifetime by addition of the surfactant SDS at the pre-stage temperature of 18 °C. Figure 4 shows the plot of the fluorescence lifetime against SDS concentration, C_{SDS}. In $C_{SDS} > 200$ mg L^{-1}, the fluorescence lifetime suddenly increased up to ca. 4.4 ns and remained constant, where the fraction of the longest component became larger. This indicates that SDS prevents the dynamic quenching at

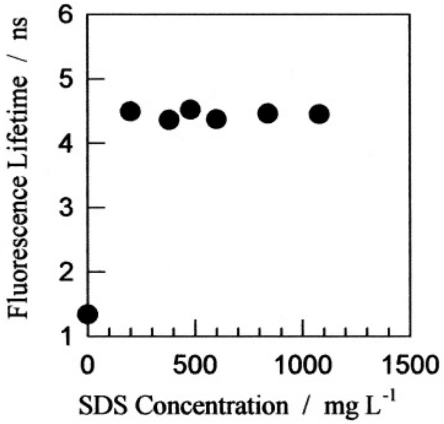

Figure 4. Plots of the fluorescence lifetime <τ> for PEVE aqueous solution (0.02 wt%) against SDS concentration, C_{SDS}. Measurements were carried out at 18 °C.

the pre-stage. That is, the dynamic quenching comes from the thermal fluctuation of segment density precedent to the phase separation. Moreover, Figure 2 shows that the fluorescence lifetime of 4.4 ns at 18 °C corresponds nearly to the value along the broken line. In other word, if the dynamic quenching were negligible, the fluorescence intensity and lifetime for PEVE aqueous solution would change monotonically and gradually with temperature similar to that for PEVE in hexane as described by the broken line in Figure 2.

F. Winnik examined the thermoreversible phase separation of aqueous solutions of poly(*N*-isopropylacrylamide) by fluorescence experiments. She monitored the phase separation by measuring electronic excitation energy transfer efficiency between donor and acceptor chromophores attached to the same polymer chain(8). The results were interpreted in terms of a mechanism of phase separation initiated by a gradual shrinking of solvated polymer coils into a collapsed state, followed by intermolecular aggregation into larger particles. The change in the energy transfer efficiency begins to occur below LCST temperature. She concluded that this might result either from a continuous decrease in the size of the polymer coil, or from an enhancement in the density fluctuation. The decrease in the size of the polymer coil will cause suppression in the chain mobility, i. e., the relaxation time will become long. Figure 2(b) shows that no decrease in the chain mobility was observed at the stage preceding the phase separation with the fluorescence depolarization. Therefore we suggest that at this stage the enhancement in the density fluctuation is the best explanation both for Winnik's work and for this study. We mean that the density fluctuation that leads to the nucleation in the phase separation may occur at the pre-stage.

Conclusion

We studied the dynamic fluorescence quenching of anthracene-labeled PEVE aqueous solution which is observed just below the phase separation temperature. In the systems without phase separation of the hexane solution of PEVE and PEVE bulk, the fluorescence lifetime decreased monotonically with the increase of temperature, but the lifetime in the latter system was about two thirds as long as that of the former. That is, in the hexane solution, no dynamic quenching occurred while in bulk, the dynamic quenching occurred at all temperatures measured. We added SDS as a surfactant to PEVE aqueous solution just below the phase separation temperature. Then, the fluorescence lifetime sufficiently increased up to the estimated value assuming no dynamic quenching. These results indicated that the dynamic quenching is induced precedent to the phase separation. That is, between 16 and 20 °C, the amplitude of thermal fluctuation of segment density becomes large and the dynamic fluorescence quenching effectively occurs through the collision between the fluorescent probe and the PEVE segments at the stage preceding the phase separation.

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