Influence of a Fluorescent Probe on the Local Relaxation Times for a Polystyrene Chain in the Fluorescence Depolarization Method

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ABSTRACT: The influence of the fluorescent probe on the fluorescence depolarization study was examined by using molecular dynamics (MD) simulation together with the fluorescence depolarization measurement. The relaxation times of the local motion, T_m, for two series of polystyrene (PS) (r-PS and a-PS), which have different molecular structures in the vicinity of the anthryl group used as a fluorescent probe, were compared. The T_m for r-PS, which has more space between the anthryl group and PS unit than a-PS, was smaller by a factor of 2 than that for a-PS, which has one methylene group as the spacer. The steric hindrance of the anthryl group with the phenyl ring of PS is considered to make the relaxation time longer. On the other hand, the activation energy may predominantly reflect the inherent chain mobility of the PS chain. The MD simulation was performed for the anthryl group-labeled PS chain with various numbers of methylene groups between the probe and styrene unit. The result explains the difference in the relaxation time between a-PS and r-PS. The T_m for the local motion of the probe-free PS segment at the chain center showed that the chain mobility of PS is slightly reduced by the introduction of an anthryl group The effect of the direction of the transition moment in the fluorescent probe for the relaxation time was also examined.

1. Introduction

The fluorescence method has been widely used for studies of the structure and dynamic properties of polymer systems.[1−4] The method has several advantages such as high microscopic sensitivity and high resolution in space and time. Therefore, one can obtain irreplaceable information using the fluorescent probe labeled at a designated position in the system. In particular, the fluorescence depolarization method provides information on the rotational relaxation of the transition moment of the fluorescent probe. The relaxation time of the probe was estimated through the measurements of the decay of the fluorescence anisotropy ratio. The local motion of polymer chain has been extensively studied for the polymers with the fluorescent probe labeled in the main chain.[5−19] We examined the local motion of polymer chains labeled with anthracene as the fluorescent probe in dilute solutions and discussed the effects of several molecular factors such as solvent condition,[13,15] molecular structure of polymer,[13−16] molecular weight,[15−18] and the position along a chain on the local chain mobility.[18] However, there still remain some questions to be solved.

First, a fluorescent probe must be used in the fluorescence method, and therefore the observed results always include the influence of the probe to some extent. Then estimation of the perturbation by the probe is necessary for analysis, but few works have been reported.[12,16,20] Pant et al. proposed the complex damped orientational diffusion model of local polymer chain motion including the effects of attached probes on the dynamics.[20] Moreover, in the fluorescence method, the molecular structure in the vicinity of the fluorescent probe is also expected to influence the observed result. The molecular structure in the vicinity of the fluorescent probe is usually controlled by the synthetic scheme. We have already synthesized sample polymers by the living anionic polymerization and subsequent coupling with 9,10-bis(bromomethyl)anthracene. The polymers were highly controlled in the molecular structure and molecular weight. By this synthetic procedure, the 9,10-dimethylanthryl group was introduced into the middle of the main chain. Recently, well-defined anthracene-labeled polystyrene (PS) was synthesized by the atom transfer radical polymerization. The obtained PS has a different molecular structure in the vicinity of anthryl group from that of the former polymer. This synthetic success enabled us to examine the effect of the molecular structure in the vicinity of the probe on the chain mobility.

Recently, molecular dynamics (MD) simulation has been utilized for the local chain dynamics study.[21−26] MD simulation gives data of coordinates of all atoms, so that we can estimate several requested properties at the atomic level. With the higher capacity of computers and with the improvement of the available force field, the experimental results can be well simulated. In the MD simulation, the molecular structure of a polymer can be modified as necessary. Therefore, the local chain dynamics of the PS chain differently labeled with anthryl groups and even that of a probe-free PS chain can be evaluated, and the influence of the probe on the

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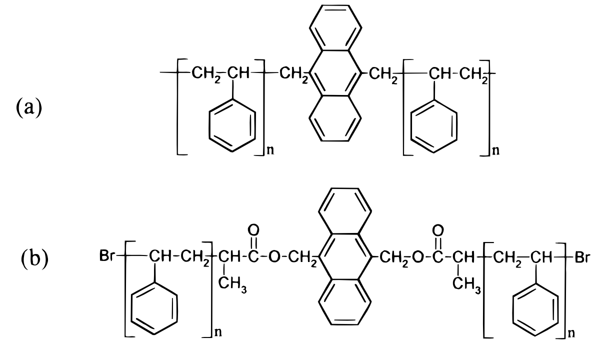

Figure 1. Molecular structures of PS labeled with anthryl group in the middle of the main chain used in the fluorescence depolarization study: (a) a-PS and (b) r-PS.

Table 1. Characterization of PS Samples Used in the Fluorescence Depolarization Study

	M_n	M_w/M_n		M_n	M_w/M_n
a-PS1	6.4	1.13	r-PS3	6.5	1.08
a-PS2	13.1	1.06	r-PS4	10.0	1.10
a-PS3	69	1.04	r-PS5	12.5	1.10
a-PS4	92	1.05	r-PS6	15.8	1.12
r-PS1	2.7	1.10	r-PS7	20	1.12
r-PS2	4.1	1.09	r-PS8	149	1.21

fluorescence depolarization study can be estimated. In addition, we can estimate the effect of the direction of the transition moment to the main chain.

In the present study, we first examined the local motion of two series of PS in dilute benzene solutions by the fluorescence depolarization method. Two series of PS chains are differently labeled with an anthryl group. We compared the relaxation times of the local motion and their molecular weight dependence for these polymers. Then, we carried out the MD simulation for both the probe-labeled PS of 20-mer and the probe-free PS of 22-mer in vacuo and compared the mobility at the chain center between these model compounds. We also calculated the rotational relaxation times of different vectors pointing in directions other than parallel to the main chain.

2. Experimental Section

A. Fluorescence Depolarization Study. *Sample Preparation.* One series of the anthryl group-labeled PS samples used in this study was synthesized by the living anionic polymerization in vacuo initiated by butyllithium, in which the living ends were coupled with 9,10-bis(bromomethyl)-anthracene. Details of the synthetic procedure have been reported elsewhere.[17,27] The incompletely coupled fraction was removed from the original sample by GPC. Figure 1a shows the molecular structure of this series of PS (a-PS), and Table 1 shows the weight-average molecular weight M_w and the number-average molecular weight M_n of a-PS.

The other series of the anthryl group-labeled PS samples was synthesized by the atom transfer radical polymerization with 9,10-bis(1-bromoethylcarbalkoxymethyl)anthracene as a bifunctional initiator. The synthetic procedure of this series of PS (r-PS) has been described elsewhere.[28] The molecular structure of r-PS is shown in Figure 1b and the characterization in Table 1.

Benzene (Dojin, spectrophotometric grade) (a good solvent) was used as a solvent without further purification. The concentration of each polymer in the sample solutions was kept below 10^{-5} M. Each solution was put into a quartz cell and degassed.

Anisotropy Decay Measurement. The measurement of time-resolved anisotropy decay was carried out by the single photon counting system. The apparatus was the same as that used in the previous studies.[16-18] The second harmonic of Ti:sapphire laser was used as a light source at 397 nm. The excitation light was vertically polarized, and the parallel and perpendicular fluorescence components to the plane of the excitation light were measured by a microchannel plate–photomultiplier tube. The fwhm of the total instrumental function was ca. 60 ps. The parallel component, $I_{VV}(t)$, and the perpendicular component, $I_{VH}(t)$, were measured alternately to avoid data distortions due to time drift. Anisotropy decay measurements were carried out in the temperature range from 10 to 30 °C.

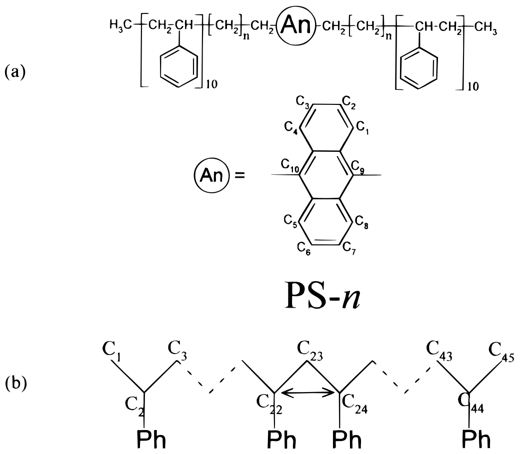

Figure 2. Molecular structures and atom numbers for PS model compound used in the MD simulation. (a) Anthryl group-labeled PS model with *n* methylene groups, PS-*n*. (b) Probe-free PS model. The arrow indicates the vector whose $C_2(t)$ was analyzed.

Data Analysis. The fluorescence anisotropy ratio, $r(t)$, is defined as

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

where G is the compensating factor and was estimated to be unity in this study. For discussion about the chain mobility, we used the mean relaxation time, T_m, which is the time integral of the reorientational autocorrelation function defined as eq 2.

$$T_m = {r_0}^{-1} \int_0^\infty r(t)\, dt \qquad (2)$$

where r_0 is the initial anisotropy ratio.

B. MD Simulation Methodology. MD simulations were carried out with a MD software, Discover 3 (Biosym Technologies),[24,25] on a CRAY Origin 2000 supercomputer in the same way as the previous MD simulation study.[26] The force field used in this study was Biosym CFF91,[29,30] which took into account bond stretching, bond angle, dihedral, improper, and their seven cross-terms. We disregarded the dielectric interaction and used a Lennard-Jones type 6–9 potential for non-bonded interactions with a cutoff distance of 6 Å. One femtosecond time steps were used in the calculation.

PS chains labeled with anthryl groups in the middle of the main chain were initially generated using Insight II (Biosym Technologies). We changed the molecular structure of the PS sample in the vicinity of the probe by inserting methylene chains between the anthryl group and styrene unit. We also generated the probe-free PS of 22-mer. The molecular structures and the atom numbers used in the MD simulation are shown in Figure 2. The molecular weight for probe-free PS is almost the same as that for PS-0 (2304 and 2286, respectively). The C_9–C_{10} vector in the anthryl group corresponds to the transition moment, L_a, of the anthryl group.[31] After 300 steps of minimization, the molecular dynamics was calculated in vacuo under a constant temperature of 300 K. The total duration time of the dynamics run was 2100 ps, and the atom coordinates used for analysis were saved every 1 ps after 100

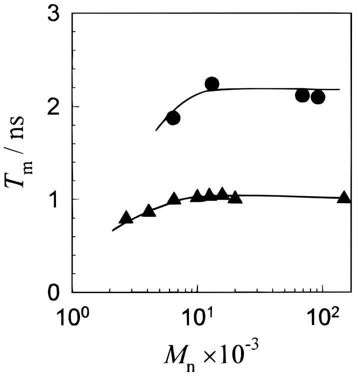

Figure 3. Molecular weight dependence of T_m for a-PS (●) and r-PS (▲) in benzene at 20 °C.

ps of discarded dynamics run. From the data of atom coordinates, we calculated the second-order orientational autocorrelation functions, $C_2(t)$, for the C_9-C_{10} vector of the anthryl group and the $C_{22}-C_{24}$ vector for the probe-free PS according to eq 3.

$$C_2(t) = \frac{\langle 3(\mathbf{u}_{mn}(0)\cdot\mathbf{u}_{mn}(t))^2 - 1\rangle}{2}$$

$$\mathbf{u}_{mn}(t) = \frac{\mathbf{r}_{mn}(t)}{r_{mn}(t)} \qquad (3)$$

where $\mathbf{r}_{mn}(t)$ is a vector pointing in the direction from the C_m to C_n atom. Then, we estimated the relaxation time with eq 2 in the same way as the fluorescence depolarization study.

To estimate the effect of the direction of the transition moment of the fluorescent probe, we also calculated $C_2(t)$ for the C_2-C_6 vector of PS-0, which is nearly perpendicular to the C_9-C_{10} vector.

3. Results and Discussion

A. Fluorescence Depolarization Study. *Relaxation Time.* Figure 3 shows the molecular weight effect on the relaxation time T_m at 20 °C for both a-PS and r-PS. The relaxation time for a-PS increases with molecular weight and reaches an asymptotic value of $T_m \cong 2$ ns at MW $\cong 10^4$, while for r-PS, the asymptotic relaxation time in MW $> 8 \times 10^3$ is ca. 1 ns. The trend that the relaxation time becomes constant above a certain molecular weight indicates that the local motion of PS in benzene measured by the fluorescence depolarization method is independent of the overall motion in higher molecular weight region. The molecular weight dependence of the relaxation time has been discussed previously.[17] Although this trend is similar for both a-PS and r-PS, the absolute magnitude of T_m for a-PS is larger than that for r-PS by a factor of 2. The critical molecular weight, M_c, at which the relaxation time becomes constant, for a-PS is also slightly larger than that for r-PS. These differences certainly result from the molecular structure in the vicinity of the fluorescent probe. By taking into account the difference in the molecular structures shown in Figure 1a,b, the cause of the difference of the relaxation times may be due to the steric effect of the styrene unit around the anthryl group. It is reasonable to consider that the steric hindrance between these two bulky units influences the relaxation time of the anthryl group. That is, the anthryl group for r-PS has longer spacers to the nearest styrene unit than that for a-PS, so that the probe of r-PS rotates more freely than that of a-PS. The observed rotational relaxation is realized by the local motion of the main chain bonds near the anthryl group, and the rotation of the anthryl group around the main chain does not contribute to the orientational relaxation of the transition moment. The result indicates that the observed relaxation time reflects the molecular structure in the vicinity of the fluorescent probe. In other words, we have to pay attention to how and where a fluorescent probe is attached to the polymer chains.

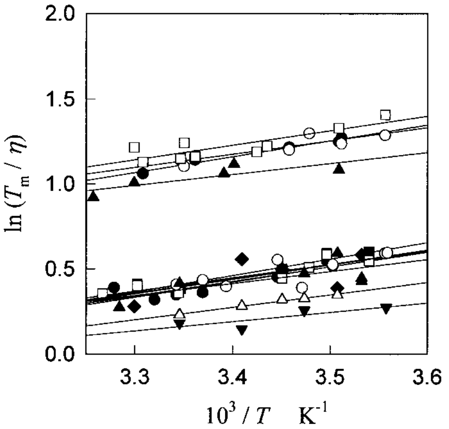

Figure 4. Arrhenius plot of T_m/η according to Kramers' theory. η is the viscosity of benzene, which is estimated from the value reported in the literature. The upper four lines are for the a-PS series: (▲) a-PS1, (□) a-PS2, (○) a-PS3, (●) a-PS4. The lower lines are for the r-PS series: (▼) r-PS1, (△) r-PS2, (▲) r-PS3, (○) r-PS4, (□) r-PS5, (■) r-PS6, (●) r-PS7, (◆) r-PS8.

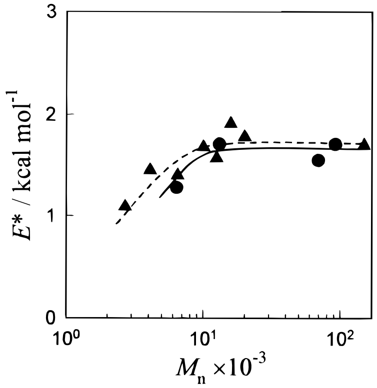

Figure 5. Molecular weight dependence of E^* for a-PS (●) and r-PS (▲).

Activation Energy. Next, we estimated the activation energy of the local motion, E^*, according to the theory of Kramers' diffusion limit, namely,

$$T_m/\eta = A \exp(E^*/RT) \qquad (4)$$

where R is the gas constant, T is the absolute temperature, η is the solvent viscosity. The solvent viscosity for benzene was estimated from the value reported in the literature,[32] e.g., 0.65 cP at 20 °C. Figure 4 shows the temperature dependence of T_m for both a-PS and r-PS in benzene. The relaxation time T_m/η, which was reduced by the solvent viscosity, η, was obtained by eq 4, and then the activation energies could be estimated from the slope of the plot in Figure 4.

Figure 5 shows the relationship between the molecular weight and the estimated activation energy in benzene. The activation energy as well as the relaxation time tends to increase with molecular weight and to become constant at MW $\cong 10^4$. The absolute magnitude of E^* ($\cong$1.7 kcal mol^{-1}) for r-PS in the saturated region

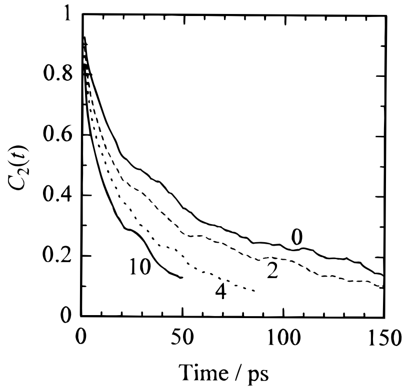

Figure 6. Decay of $C_2(t)$ for PS-*n* by the MD simulation. The numbers shown in the figure indicate the number of methylene groups, *n*.

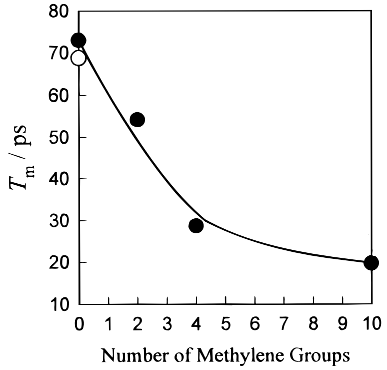

Figure 7. Influence of the number of methylene groups on T_m. Unfilled circle (○) indicates T_m for the probe-free PS chain.

is almost the same as that for a-PS, although the relaxation time is markedly different. The obtained activation energy tends to reflect the higher barrier height. That is, we assume that the activation energy reflects the inherent higher energy barrier in the styrene sequence for both a-PS and r-PS.

B. MD Simulation. Figure 6 shows the decay of the autocorrelation function $C_2(t)$ for the C_9-C_{10} vector of PS-*n* (Figure 2a) labeled with anthryl groups having various numbers of methylene groups as a spacer. The more methylene groups the PS chain has, the shorter the relaxation time is. Figure 7 shows the relationship between the number of methylene groups and the relaxation time of the anthryl group. With the increase in the number of methylene groups up to 4, T_m decreased by a factor of ca. 2.5. The number of bonds between the anthryl group and styrene unit for r-PS corresponds to that for PS-4, and the difference in the relaxation time of PS-4 from that of PS-0 is in agreement with the difference in the relaxation time of r-PS from that of a-PS.

The unfilled circle in Figure 7 shows the relaxation time of the probe-free PS chain (Figure 2b). The T_m of the probe-free PS chain is slightly smaller than that of the PS-0 chain. The result shows that the chain mobility may not be influenced so much by the introduction of anthryl group as the fluorescent probe to the chain center of PS. The molecular weight of the anthryl group (=176) is larger than that of the styrene monomer unit (=104), but one of the rings of the anthryl group stands with the interval of three main chain carbon atoms from a neighboring phenyl group of styrene unit like the normal styrene sequence. Therefore, the steric hindrance between the anthryl group and styrene unit is considered to be similar to that between adjacent styrene units, and the relaxation times are not much different. If this is the case, the local motion of polyethylene (PE), which is considered to be more flexible than PS, is expected to be more influenced by the introduction of an anthryl group. The T_m was 2.4 ps for the probe-free PE chain but increased to 4.3 ps for the anthryl group-labeled PE chain as expected.

Table 2. Comparison of the Relaxation Times for Vectors Pointing Different Directions

	C_2-C_6	C_9-C_{10}
T_m/ps	41	73

Table 2 shows the influence of the direction of the transition moment of the anthryl group, which was used as the fluorescent probe, on the observed relaxation time. The relaxation time T_m of the C_2-C_6 vector in the anthryl group is smaller than that of the C_9-C_{10} vector. This is due to the relaxation by the rotation around the main chain, and this rotational mode has no effect on the relaxation of the C_9-C_{10} vector. Consequently, the direction of the transition moment of the fluorescent probe is also important in the fluorescence depolarization study. The relaxation time of the C_9-C_{10} vector is longer than that of the C_2-C_6 vector, and the C_9-C_{10} vector represents the mobility of the vector along the main chain bond as well.

4. Conclusion

We examined the effect of the fluorescent probe and the structure around the probe in the fluorescence depolarization analysis by using MD simulation. The absolute magnitude of T_m for r-PS, which has a longer flexible spacer between the anthryl group and PS unit, is smaller by a factor of 2 than that for a-PS. This shows that the relaxation time is primarily influenced by the structure around the probe.

Next, we carried out the MD simulation for various distances between the anthryl group and styrene unit separated by a flexible methylene group. As the number of methylene groups increased up to four, T_m decreased by a factor of 2.5, in agreement with the fluorescence depolarization study. We also simulated the local motion of the probe-free PS chain and compared the relaxation time at the chain center with that of anthryl group-labeled PS. The chain mobility of PS slightly changed with the introduction of an anthryl group. The relaxation time of the vector that is nearly perpendicular to the direction of the transition moment of the anthryl group is considerably shorter than that of the vector corresponding to the transition moment, L_a, which represents the chain mobility of the vector along the main chain bond.

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