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Local Chain Dynamics of Adsorbed Polystyrene Studied by Time-Resolved Fluorescence Anisotropy

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Introduction. The study of adsorbed polymers has dynamic and equilibrium aspects. The equilibrium aspects are best understood; there is a rich understanding, theoretical and experimental, regarding such matters as the amount adsorbed under various environmental conditions and the equilibrium conformational distribution profile of layers adsorbed from solution and melt.[1] Dynamic aspects have received less attention and are more problematical, partly because of the paucity of experimental methods that have sufficient sensitivity to probe time-dependent changes within molecularly thin layers. One approach used recently is to study the exchange dynamics between the adsorbed state and free solution,[2,3] but this is restricted to long times, minutes to hours, and is not sensitive to relaxations within single polymer chains. Another approach is to study collective rearrangements as revealed by evanescent wave light scattering,[4,5] but this designed to study collective fluctuations on the order of the optical wavelengths. Still other approaches involve nuclear magnetic resonance (NMR) and electron spin resonance (ESR).[1] These methods have the advantage of probing more rapid relaxations, characteristic of individual polymer chains during the time that they reside in the adsorbed state. But their interpretation is generally model-dependent, and these methods do not give direct quantitative dynamic information such as the characteristic times for translational or rotational motion of individual segments within adsorbed chains.

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In this study we present, for the first time to be best of our knowledge, direct measurements of the rotational time scale of chain segments located within loops along adsorbed flexible polymer chains. The method was time-resolved fluorescence anisotropy following two-photon excitation. In related studies using this method, Yamamoto and co-workers studied extensively the local chain dynamics of synthetic polymers in different isotropic environments of bulk dilute solution. They showed the respective effects of the location of the label molecule attached to the end of chain[6] or to the center of chain,[7] the effect of a spacer next to the label,[8] the effect of molecular weight,[9] and the effect of surface pressure in a Langmuir−Blodgett monolayer.[10] Ediger and co-workers have studied reorientation dynamics in a melt environment.[11] Although there have been reports related to the restricted motion of labeled polyelectrolyte adsorbed on colloid silica,[12] two-dimensionally adsorbed layers do not appear to have been studied previously, perhaps because of the significant photobleaching problem that results when slow translational diffusion causes fluorescent probes to reside for an exceptionally long time within the zone illuminated by the probing laser beam. Here we present a new approach to alleviate this problem and our initial experimental results using it. The main point was to measure both the vertically and horizontally polarized fluorescence at the same time, using two PMTs (photomultiplier tubes) rather than a single one, while at the same time normalizing reliably for the different sensitivity and background noise of these detectors.

Experimental Section. a. Materials. Anthracene-labeled polystyrene (Polymer Source, Inc., Québec, Canada) and spectroscopic grade cyclohexane (Aldrich) were purchased and used without further purification. The anthracene was located at the center of chains with weight-average molecular weight 106 000 g mol^{-1} and ratio of weight-average to number-average molecular weight $M_w/M_n = 1.04$. The transition moment of anthracene is perpendicular to the molecular axis (i.e., parallel to the backbone of the polystyrene chain) as

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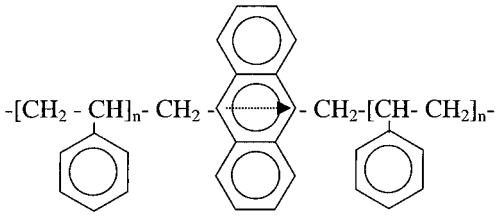

Figure 1. Schematic diagram of the anthracene-labeled polystyrene. The anthracene was located in the center of the polystyrene chain. Its transition moment (shown by arrow) was vertical to the molecular axis of anthracene, i.e., along the chain backbone.

shown in Figure 1. The maximum excitation and emission wavelengths were found to be 381 and 433 nm, respectively. The fluorescence lifetime of anthracene is 4.7 ns in bulk cyclohexane solution.[13] Control experiments, in which anthracene was dissolved in cyclohexane at concentrations up to 1 μM, showed that anthracene itself did not adsorb to quartz.

b. Instruments. Two-photon excitation was achieved using a femtosecond Ti:sapphire laser (Mai Tai, Spectra-Physics) whose fwhm (full width at half-maximum) pulse was measured to be 100 fs. The repetition rate was 80 MHz, and the wavelength was 800 nm.

Excitation of the anthracene probe was performed within a homemade microscope designed to combine this measurement with the surface forces apparatus (SFA). However, a cylindrical quartz cell was used in these initial experiments in order to simplify the surface geometry. In the design that we employed, the vertically polarized laser beam was first split into two beams, and one of them was introduced into an objective lens (Mitutoyo, N.A. = 0.55) and focused onto the sample. The other beam was used as a trigger signal for the single photon counting system (Becker & Hickl GmbH, Berlin, Germany). The emitted fluorescence was collected by the same objective lens and focused again by a tube lens in order to increase the response of the photomultiplier tube (PMT) detectors.

The adsorbed molecules were found to be exceptionally vulnerable to photobleaching, and time drift of the fluorescence intensity was a serious problem in our initial experiments in which the polarization was modulated with detection using a single photodetector. Instead, we adopted the approach of measuring fluorescence in orthogonal polarization directions simultaneously. A fast PMT (Hamamatsu, R5600) and a photodiode were used to detect the fluorescence and the trigger signal, respectively. The PMT signal was input to the time-to-amplitude converter as a start signal followed by a constant fraction discriminator (Becker & Hickl GmbH, TCSPC730). In this setup the total instrument response function was around 150 ps (PMT1) and 240 ps (PMT2) for the two detectors.

c. Sample Preparation. The anthracene-labeled polystyrene was dissolved in cyclohexane to a final concentration of 900 nM (0.1 mg mL^{-1}). Cyclohexane is a poor solvent for polystyrene at the room temperature of 24 °C, with the Θ temperature equal to 34.5 °C. After cleaning the quartz cell by acid bath in the manner described previously,[14] the solution was introduced into the 0.5 cm diameter cylindrical quartz cell, and 30 min was allowed for adsorption. After rinsing out the polystyrene with pure cyclohexane several times, it was refilled with pure cyclohexane. At the beginning and end of each experiment, the fluorescence of bulk cyclohexane was measured, and no fluorescence was detected above the background level. This signifies that no adsorbed polystyrene dissolved into the solution.

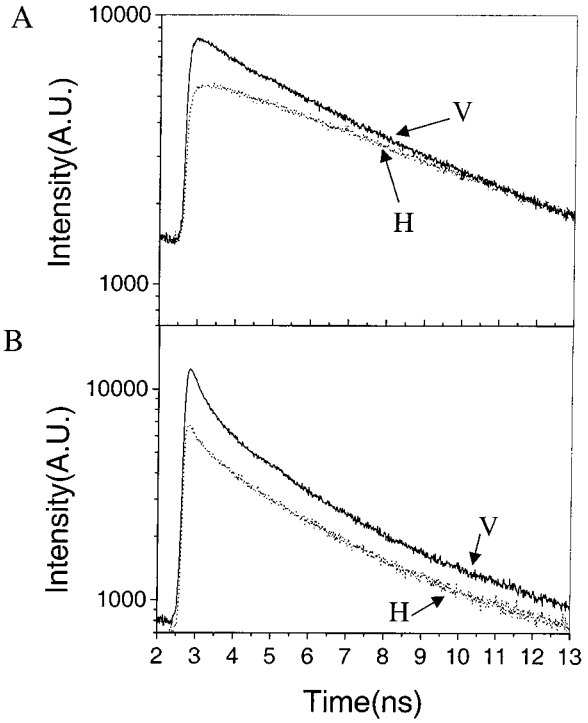

Figure 2. Fluorescence intensity decay of anthracene-labeled polystyrene in bulk cyclohexane solution (panel A) and adsorbed polystyrene (panel B). Polarized intensity in orthogonal directions (V, vertical polarization; H, horizontal polarization) is plotted semilogarithmically against time on the nanosecond time scale. The data overlapped rapidly in bulk solution, indicating rapid rotational diffusion, but the surface decay was slower. All measurements were performed at room temperature.

Results and Discussion. Figure 2A shows the time-resolved fluorescence of anthracene-labeled polystyrene in dilute cyclohexane solution, after calibrating the photodectors by the method described below. Fluorescence intensities of vertical and horizontal emitted fluorescence are plotted semilogarithmically against time on the nanosecond time scale. Since the polystyrene chains rotated relatively freely, the two polarized fluorescence traces overlapped soon after excitation. However, when the polystyrene was allowed to adsorb onto the quartz surfaces, rotational motion was hindered, and the two polarized components failed to overlap for a considerably longer time, as shown in Figure 2B.

Since anthracene comprised a rigid portion of the chain backbone, rather than being pendant to it as are many other fluorescent probes, its anisotropy decay gave direct information about the local dynamics of the chain backbone. Anisotropy, r, is defined as

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

where G is a compensating factor for the two-photon detectors, I is intensity, the first subscript refers to the laser polarization, and the second subscript refers to the direction of fluorescence polarization. For example, I_{VH} is the intensity of horizontally polarized fluorescence excited by a vertically polarized laser beam.

In the case of adsorbed polystyrene, the fluorescence intensity degraded rapidly after illumination. This effect was probably exacerbated by the two-photon excitation (one-photon excitation is milder), but two-photon excitation is required for our ultimate purpose of spatially

addressing the fluorescence signal within the surface forces apparatus down to a micron-sized spot. The observed decay of photon counts appeared to reflect some combination of reversible photobleaching[15] in addition to the irreversible photodegradation, because the photon counts were observed to recover partially after illumination was stopped. It was not possible to measure I_{VV} and I_{VH} in the traditional way of rotating a polarizer between two orthogonal polarizations.

Instead, we split the emitted fluorescence into two beams using a polarizing beam splitter and measured the intensity of the two signals simultaneously using two detectors. A troublesome issue became that the two detectors differed in their time response, background level, and sensitivity. It is true that time response differences can in principle be corrected by deconvolution of the instrument response function, that sensitivity differences can in principle be adjusted by using a reference sample, and that background differences may also be calibrated and adjusted. But the complexity of these many corrections made the final result difficult to trust, and the following simpler approach was developed.

To calibrate the two detectors, PMT1 and PMT2, vertically polarized fluorescence (I_{VV1}) and horizontally polarized fluorescence (I_{VH2}) from the bulk were measured at the same time using both detectors, and then horizontally polarized fluorescence (I_{VH1}) was measured with PMT1 by rotating the polarizing beam splitter. The number after V or H refers to the PMT identification number. Because rotation of dye molecule in bulk solution was very fast and there was no significant photobleaching, the tails of I_{VV1} and I_{VH1} matched as shown in Figure 1A. If the laser power is assumed to be constant, the relation between the bulk and surface fluorescence signals is

$$\frac{\int_0^t I_{VV1}\,dt}{\int_0^t I_{VH1}\,dt} : \frac{\int_0^t I_{VV1}\,dt}{\int_0^t I_{VH2}\,dt} = \frac{\int_0^t I'_{VV1}\,dt}{S} : \frac{\int_0^t I'_{VV1}\,dt}{\int_0^t I'_{VH2}\,dt} \quad (2)$$

where I's are the fluorescence intensity for the adsorbed polystyrene molecules defined same as the bulk. The symbol S is the integrated area of the horizontally polarized fluorescence (I'_{VH1}) from the adsorbed polystyrene measured by PMT1.

Since all the variables are measured except S, S can easily be calculated. If this value of S is compared with its separate measurement using PMT1 of the horizontally polarized fluorescence from the adsorbed polystyrene, we obtain the numerical ratio that should be multiplied by the measured value to produce equality in eq 2. In this experiment this was 1.48, and the result is shown in Figure 2B. The validity of this approach was confirmed in two ways. First, the two ratios on the left-hand side of eq 2 (bulk) were measured repeatedly and found to be nearly constant, ±2%. It is reasonable to assume this ratio was also constant on the right-hand side of eq 2 (surface). Second, the peak value of I'_{VH1} was calculated by replacing the integration in eq 2 by the peak values. This was compared with the peak value calculated from eq 2, and the values agreed within 2%.

Figure 3 compares the anisotropy decay of labeled polystyrene after making this normalization and shows that the decay of anisotropy of adsorbed molecules was significantly retarded. Fluorescence anisotropy is plotted against elapsed time on the nanosecond time scale. Note also that while it is true that there may exist

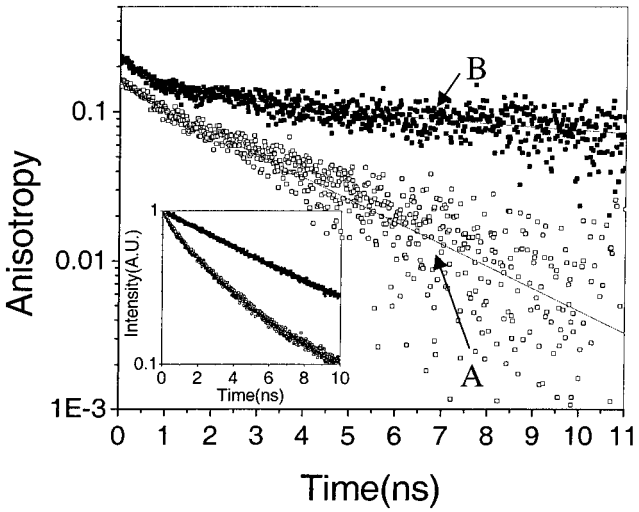

Figure 3. Time-resolved anisotropy decay computed from the data in Figure 2. The curve denoted A indicates dilute solution. The curve denoted B denotes the adsorbed state. Inset shows the lifetime of bulk (upper curve) and adsorbed polystyrene (lower curve). All curves are plotted semilogarithmically against time on the nanosecond time scale.

relaxation modes too rapid to be resolved with our instrument, the initial value of the measured anisotropy, $r(t\rightarrow 0)$, was larger for adsorbed chains than for chains in solution, indicating that adsorbed polystyrene had a net anisotropy.

Since the probe was located in the middle of the polystyrene chain, it appears likely that it could exist only within the loops of adsorbed chains. Tails are necessarily toward the end of the molecule, so this possibility can be discounted. Trains require physical contact with the surface, but our control experiments showed that anthracene alone did not adsorb. Since the transition moment of anthracene is vertical to its molecular backbone, as sketched in Figure 1, rotation around the backbone axis does not affect the anisotropy. Only twisting of loop in the adsorbed chain structure can change it. Furthermore, if the loop were long enough, the probe orientation dynamics would be indistinguishable from the bulk. Therefore, the observed large value of anisotropy as $t \rightarrow 0$ implies relatively short loops. In future work it would be desirable to quantify this statement.

The inset of Figure 3 shows the lifetime decay for each case. Bulk dilute solution shows single-exponential decay, but the case of adsorbed polystyrene shows a second-order decay. The lifetime decrease upon adsorption may reflect quenching or nonradiative energy transfer between the nearby dye molecules, and it implies the concentration of the adsorbed polystyrene is higher than that in bulk.

To quantify the anisotropy decay, the data were fitted in the customary manner[7] with the sum of independent double-exponential decays:

$$r(t) = r_0\{x \exp(-t/\tau_1) + (1 - x) \exp(-t/\tau_2)\} \quad (3)$$

and the mean relaxation time,[7] τ_m, was calculated:

$$\tau_m = r_0^{-1}\int_0^\infty r(t)\,dt \quad (4)$$

$$\tau_m = x\tau_1 + (1 - x)\tau_2 \quad (5)$$

The results of this fit (amplitudes and decay times) are

Table 1. Fitting Parameters for Anisotropy and Lifetime Measurements

	anisotropy		lifetime	
	bulk	surface	bulk	surface
amplitude 1 (AU)	0.88	0.54	6400	3100
τ_1 (ns)	2.9	7.8	6.8	3.3
amplitude 2 (AU)	0.12	0.46		800
τ_2 (ns)	0.28	0.52		0.48

shown in Table 1. The mean rotational relaxation times are 2.6 and 4.4 ns for bulk and adsorbed polystyrene, respectively. Since the local motion of the fluorescence probe consists of the combination of various motional modes, anisotropy can be represented by a series of exponential decay functions. However, Horinaka et al.[7] empirically concluded that double-exponential function is enough to represent the anisotropy because there is not a specific model to describe the local motion of polymer chain successfully.

It is interesting that the same double-exponential decay function describes equally well the surface and bulk fluorescence decays. One might have expected to find a large amount of heterogeneity at the surface owing to the expected[1] distribution of loop sizes, which would have been reflected in the need to employ, to describe the surface response, a more flexible fitting function than just the sum of two exponentials. Our null result in this respect may signify that the slower anisotropy decay observed at the surface reflects, more simply, the enhanced local concentration of nearby polymer segments.

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