Journal of Fluorescence, Vol. 8, No. 3, 1998

Fluorescence Depolarization Due to Excitation Energy Migration in Polymeric Micelles: A Monte Carlo Study[1]

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Received July 13, 1997; revised June 3, 1998; accepted June 3, 1998

Cores of block copolymer micelles have been studied by Monte Carlo simulation. Core-forming chains have been modeled as self-avoiding chains enclosed in a spherical cavity and tethered to its surface. A fraction of the untethered end segments of chains (18–53%) has been treated as fluorescent probes. The time-dependent solution of the Pauli master equation that describes excitation energy migration among probes has been averaged in an ensemble of 10^4 simulated cores. We have studied the dependence of the depolarization function $G^s(t)$, i.e., the probability that the originally excited probe is still excited at time t, on the chain length and on the energy migration critical radius of the probe. Cores with randomly solubilized probes and with clusters of probes have been also studied for comparison.

KEY WORDS: Time-resolved fluorescence depolarization; excitation energy migration; block copolymer micelles; tethered chains; Monte Carlo

INTRODUCTION

Spherical multimolecular micelles represent one type of copolymer structure that has been attracting the interest of many research teams in recent years. They exist in dilute solutions in selective solvents (thermodynamically good solvents for one type of block and nonsolvents for the other). Properties of micellizing systems have been studied both theoretically and experimentally [1]. Fluorescence techniques have proved to be powerful tools for studying such systems. These studies have led to a fairly good understanding and phenomenological description of the micellization process.

To be able to interpret our fluorometric measurements [2] on block copolymer micelles containing end-tagged insoluble blocks, we have undertaken a series of computer-based Monte Carlo simulations of tethered chain conformations under conditions when the tethered chains mimic the core-forming blocks. In our earlier studies [3] we have presented various functions describing chain conformation. In this communication, we present simulated data on excitation energy migration in modeled micellar cores.

METHOD

The time-resolved fluorescence anisotropy, $r(t)$, for vertically polarized excitation pulses is defined by the following equation [4]:

$$r(t) = [F_v(t) - F_h(t)]/[F_v(t) + 2F_h(t)] \quad (1)$$

where $F_v(t)$ and $F_h(t)$ are the vertically and horizontally polarized time-resolved fluorescence emissions, respectively. In micellar systems with tagged core-forming blocks, the anisotropy monitors the complex rotational

[1] This paper is based on the communication at the 2nd Conference on Fluorescence Microscopy and Fluorescent Probes, Prague, Czech Republic, 9–12 April 1997.

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1053-0509/98/0900-0207$15.00/0

diffusion-like motion of pendant fluorophores in micellar cores and the excitation energy migration (EEM) among fluorophores which are (at least some of them), located relatively closely to each other. Rotational diffusion of whole micelles occurs on an incomparably longer time scale than fluorescence decays of typical fluorophores, and this slow motion is therefore not observed by the time-resolved fluorescence measurements. In very selective solvents (e.g., block polyelectrolyte micelles in aqueous media [2a, b]), the rotational motion of pendant fluorophores embedded in compact and rigid micellar cores is frozen, and its contribution to the fluorescence depolarization is negligible.

Processes of time-resolved fluorescence depolarization due to energy migration have been theoretically studied by several authors[5], and it has been shown that the time-resolved fluorescence anisotropy may be generally expressed by the following expression [5g]:

$$r(t) = r_0(t = 0)\,[aG^S(t) + b] \tag{2}$$

where the first term, $r_0(t = 0)$, describes the initial anisotropy at the instant of excitation. The initial anisotropy depends on mutual orientation of transition dipole moments of absorption and emission and, for a given fluorophore and a given wavelength, is constant. The second term, $G^S(t)$, reflects the excitation energy migration. $G^S(t)$ describes the ensemble average probability that at time t, the excitation is localized at the fluorophore that was originally excited at time $t = 0$. Constants a and b depend on the system studied. The numerical value of b is usually fairly low compared with a, and its contribution may be neglected in a system of disordered frozen fluorophores [5g,h].

Compared with the excitation energy transfer from a fluorophore to traps, theoretical treatment of the excitation energy migration among chemically identical fluorophores is more difficult. One of the first papers on this topic was the work of Haan and Zwanzig [5a]. Later, a cumulant expansion method, which provides a general solution of EEM among fluorophores in infinite volume, was developed by Huber [5b], and Fayer *et al.*[5c–e] published solutions of EEM for several finite geometries. Our computer-aided stochastic approach allows for numerical evaluation of the experimentally available fluorescence anisotropy decay. This average decay is calculated on the basis of a fairly large ensemble of solutions of the equation that describes energy migration in individual simulated micellar cores. Computer simulations were performed in three steps.

(i) Micellar cores are simulated on a tetrahedral lattice (with the lattice distance l) according to a procedure described in earlier papers[3]. As soon as one equilibrated micellar core with N chains of length L is created, $N_F \leq N$ chain ends are randomly "labeled" by fluorophores. Only one fluorophore is "excited," which corresponds to usual experimental conditions.

(ii) The master equation that describes excitation energy migration [5a] was solved for a great number of simulated configurations $K(\mathbf{r}_1,\ldots,\mathbf{r}_{N_F})$ of N_F fluorophores,

$$dP_j(K,t)/dt = -P_j(K,t)/\tau_0 + \Sigma_i w_{ij}\,[P_i(K,t) - P_j(K,t)] \tag{3}$$

$P_j(K,t)$ is the probability that for a given configuration $K(\mathbf{r}_1,\ldots,\mathbf{r}_{N_F})$, the excitation is localized at the jth fluorophore at time t, w_{ij} is the energy transfer rate between fluorophores i and j ($w_{jj} = 0$), and τ_0 is the fluorescence lifetime of the donor in the absence of traps. For the dipole–dipole interaction, the excitation energy transfer rate is expressed by the Förster formula [4]

$$w(r_{ij}) = (1/\tau_0)(R_0^{FF}/r_{ij})^6 \tag{4}$$

[6], where r_{ij} stand for mutual distances between fluorophores i and j of the same type. The Förster radius, R_0^{FF}, is the distance for which the depletion rates of the excited state by energy transfer and by fluorescence are equal. The Förster radius, R_0^{FF}, depends on the mutual orientation of two fluorophores and is given by the formula [6] $R_0^{FF} = \sqrt[3]{\kappa}R_0$, where R_0 is the critical radius and κ is the orientation factor. In the system of randomly oriented fixed fluorophores, the average value of κ^2 is $\langle\kappa^2\rangle = 0.476$ [6]. The first term of the r.h.s. of Eq. (3) describes the depletion rate of the excited state by various radiative and nonradiative processes, and the sum of differences $\Sigma_i w_{ij}[P_i(K,t) - P_j(K,t)]$ describes the process of excitation energy migration. The set of Eqs. (3) was transformed into a set of linear equations [using the Laplace transform of $P_{ij}(K,t)$ functions] and solved numerically.

(iii) The time-dependent probabilities that excitation at time t is located on the initially excited fluorophore are averaged in an ensemble of ca. 10^5 individual micellar cores, and the experimentally observable average function, $G^S(t)$, is obtained. Since the creation of one equilibrated micelle is considerably more time-consuming than the numerical solution of the set of Eqs. [3], several fluorophores in one equilibrated micellar core were successively "excited" at random. This modification accelerates the simulation procedure and improves the statistics of simulated data.

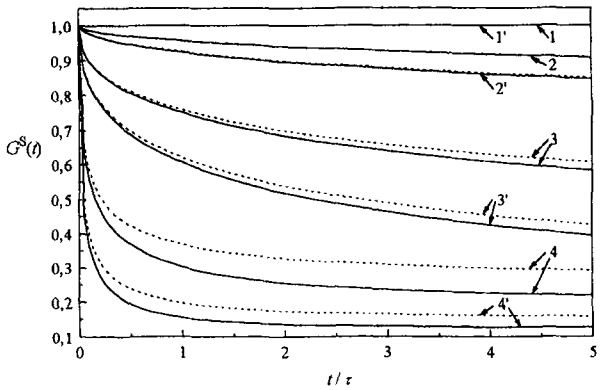

Fig. 1. Depolarization function, $G^S(t)$, for micellar systems (15 tethered chains of length 112 segments constrained in a sphere of radius $R = 10l$ and average segment density $\langle g_s \rangle = 0.6$) with a Poisson distribution function (PDF) (dashed lines) and a fixed number of fluorophores (FNF) (solid lines) for various critical radii: $R_0 = 0.5l$ (1), $2.5l$ (2), $5.0l$ (3), and $10.0l$ (4). The average and fixed number of fluorophores is five (marks without a prime) and eight (marks with a prime), respectively.

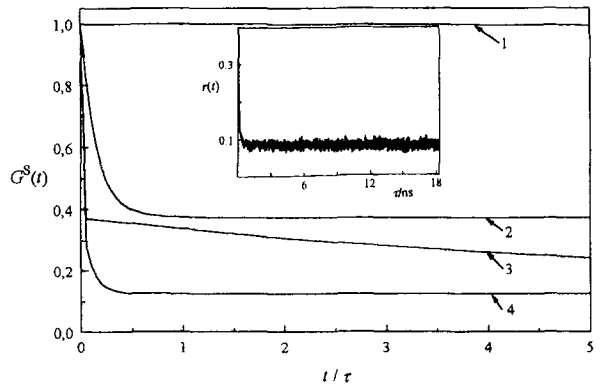

Fig. 2. Depolarization function, $G^S(t)$, for systems with three clusters of fluorophores. There are eight fluorophores [$R_0 = 0.5l$ (1), $2.5l$ (2), $5.0l$ (3), and $10.0l$ (4)] in the micellar core, distributed in two "trimers" and one "dimer." The radius of the spherical core is $10.0l$. Inset: Experimental time-resolved fluorescence anisotropy, $r(t)$ (Procházka, K., Kiserow, D., and Webber, S. E., unpublished experimental data), for mixed polystyrene–*block*-poly(methacrylic acid) micelles with 10% of end-tagged polystyrene blocks in aqueous buffer, pH 9. Concentration of copolymer, $c = 5.10^{-3}$ g/ml; excitation at 298 nm; emission at 314 nm. Details of the experimental setup described in Refs. 2a and c.

RESULTS AND DISCUSSION

In fluorometric studies of excitation energy migration in organized systems, it is often necessary to vary the average concentration of fluorophores in the system. In experimental fluorometric studies on block copolymer systems, the concentration of fluorophores in micelles may be varied by mixing tagged and nontagged copolymer samples. We assume that the structure of mixed micelles does not depend on the ratio of tagged-to-nontagged chains. This assumption is reasonably fulfilled if both copolymer chains are chemically identical and do not differ in the length of blocks, if the size of fluorophores does not differ considerably from that of polymer segments, and if the chemical nature of fluorophores precludes large differences in interactions among fluorophores, segments, and solvent molecules. In order to get the theoretical background for the interpretation of our earlier experimental data [2], we have been studying two types of systems: (i) systems with fixed numbers (FNF) of end-attached fluorophores, N_F, in all micelles and (ii) systems with a Poisson distribution of fluorophores among micelles (PDF), with an average number of fluorophores N_F.

A comparison of depolarization functions $G^S(t)$ for systems with a fixed number of fluorophores (FNF; solid lines) and with a Poisson distribution of fluorophores (PDF; dashed lines) is shown in Fig. 1. The figure shows that, first, differences between corresponding curves increase with increasing critical radius and, second, the higher is N_F, the smaller is the difference in residual values of $G^S(t)$. Since equations that describe the time-resolved depolarization of fluorescence are strongly nonlinear in N_F, it is impossible to discuss the effect caused by the Poisson distribution of fluorophores among micelles in a straightforward manner. However, we show in the Appendix that the residual value of the depolarization function $G^S(t)$ at long times is always higher in PDF than FNF systems. Simulated data show that recalculation of the spatial distribution of fluorophores from experimental anisotropy decays is an ambiguous problem that requires reliable additional information.

Fluorescence anisotropy decays that we measured for some micellar systems with end-tagged insoluble blocks [2a, c] differ significantly from simulated decays. An example is shown in the inset in Fig. 2 (Procházka, K., Kiserow, D., and Webber, S. E., unpublished data). We believe that this type of anisotropy decay curve may be caused by clustration of fluorophores due to favorable fluorophore–fluorophore interactions. To support this hypothesis, we have calculated the $G^S(t)$ function for a predefined model system of three clusters. The configuration of fluorophores is the following: two clusters consisting of three fluorophores and one cluster of two fluorophores form an equilateral triangle centered at the center of a micellar lattice. The distance between the core center and the center of each cluster is $4.0l$; the

distance between fluorophores in each cluster is $2\sqrt{2/3}l$ Figure 2 shows the $G^S(t)$ functions for several values of R_0. In a restricted system (i.e., one micellar core) containing a limited number N_F of randomly oriented fluorophores with a critical radius comparable with the core radius (curve 4), energy migration proceeds among all fluorophores very rapidly and a nondepleted fraction of the excitation energy is distributed uniformly among all fluorophores at long times after excitation. The residual value of the $G^S(t)$ function equals $(1/N_F)$th part of its original value (0.125). For low values of the critical radius (significantly lower than the intercluster distance), energy migration occurs within individual clusters only (curve 2), and the fast initial decay is followed by a high constant anisotropy during quite a long time interval till the complete depletion of the excited state of the fluorophore. In the model system, the residual value of $G^S(t)$ function equals the expected value 0.375 [it is calculated as the sum of probabilities that a given fluorophore is excited, multiplied by the residual anisotropy of the cluster in which the fluorophore is treated:$(3 \cdot {}^1/_3 + 3 \cdot {}^1/_3 + 2 \cdot {}^1/_2) = 8$]. Curve (3) depicts the case when the value of R_0 allows very fast depolarization within individual clusters and fairly limited energy transfer between clusters. The initial drop in $G^S(t)$ is very fast due to depolarization within clusters to the value 0.375, and then a slow decrease follows due to intercluster communication. The most striking feature of the depolarization curve is the sharp transition between the two time regimes.

The comparison of experimental and simulated curves suggests that the high residual anisotropy may be caused by a clustering of fluorophores. Despite the fact that the last system studied was artificially constructed, it gives a good example of what may happen in fluorescence anisotropy measurements on real micellar systems, and it helps in understanding experimental depolarization curves.

SUMMARY

1. Diffusive processes of excitation energy migration in dense polymer systems with fluorophores attached at the ends of self-avoiding tethered chains enclosed in small spherical volumes have been studied by computer simulations. Constrained systems of tethered chains have been used as models of cores of block copolymer micelles in selective solvents.

2. The time-resolved depolarization function, $G^S(t)$, which describes the time-resolved fluorescence anisotropy decay due to energy migration among fluorophores, has been calculated for systems with fixed numbers of fluorophores in all micelles and for systems with a Poisson distribution of fluorophores among micelles.

3. It has been found that fluorescence anisotropy decays for systems differing in the number distribution of fluorophores among individual micelles in the system are experimentally discernible, however, differences between individual curves are not important.

4. It has been shown that potential clustering of fluorophores in micellar systems may strongly affect the anisotropy decays and cause a high residual anisotropy.

APPENDIX

The purpose of this Appendix is to show that the residual anisotropy in a system with a fixed number of fluorophores is lower than in a system with a Poisson distribution of fluorophores among individual micelles in the system.

In a macroscopic sample of micelles (each micelle consists of N_{max} chains) with a fixed number of μ fluorophores in the core, the limiting residual value of the depolarization function is $G^S(t) = 1/\mu$. In a system with a Poisson distribution of fluorophores (with an average number of fluorophores μ), each core containing N fluorophores contributes to the ensemble average by the value $1/N$. We want to show that the following relation is fulfilled for $\mu \geq 2$:

$$\left(\sum_{N=1}^{N_{max}} \frac{e^{-\mu}\,\mu^N}{N!} \cdot \frac{1}{N} \Big/ \sum_{N=1}^{N_{max}} \frac{e^{-\mu}\,\mu^N}{N!}\right) > \frac{1}{\mu} \qquad \text{(A1)}$$

After simple rearrangements we get

$$\frac{\mu}{2} + \sum_{N=2}^{N_{max}} \frac{\mu^N}{N!} \cdot \frac{1}{N(N+1)} > 1 \qquad \text{(A2)}$$

Since the second term in the left-hand-side of relation (A2) is positive, the relation is fulfilled for $\mu \geq 2$.

ACKNOWLEDGMENTS

This work was partially supported by Grant VS97 103 (Ministry of Education of the Czech Republic) and by Charles University Grant No. 0188/1997.

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