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On the Intramolecular Excimer Formation of 1,10-Bis(1-Pyrene)Decane in Organized Media

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The formation of an intramolecular excimer of 1,10-bis(1-pyrene)decane in sodium dodecyl sulfate (SDS)/bovine serum albumin (BSA) and SDS/poly(ethylene oxide) (PEO) solutions was studied by steady-state and time-resolved fluorescence methods to determine the effect of viscosity. The viscosity-dependent ratio between intramolecular excimer and monomer fluorescence intensities of 1,10-bis(1-pyrene)decane was obtained by steady-state fluorescence measurements. The results show that in micelle-like clusters formed in SDS/BSA and SDS/PEO systems, the microviscosity is higher than in free micelles. It was found that Birks' kinetic model was not valid in this case, and the monomer and excimer fluorescence decays had to be fitted by a sum of three exponentials. The excimer formation kinetics in constrained systems is more complex than in homogeneous solutions, but it is possible to find an empirical parameter depending on viscosity.

KEY WORDS: Intramolecular excimer; microviscosity; fluorescent probe method; micelles; 1,10-bis(1-pyrene)decane.

INTRODUCTION

It is well known that the derivatives of di[1-pyrenyl]alkanes, $Py(CH)_2)_nPy$, may form intramolecular excimers whose fluorescence is sensitive to the viscosity of the medium [1,2]. The I_E/I_M ratio (I_M = the intensity of monomer fluorescence, I_E = the intensity of excimer fluorescence) is used as a viscosity measure, the value of which is calibrated using "calibration solutions" (mixtures of glycerin–ethanol or hexadecane–liquid paraffin).

Application of this method for determination of the "microviscosity" of micelles showed that the results varied as a function not only of the values of *n* (e.g., Ref. 6 or 9), but also of the different sets of calibration solutions used [3]. The use of other fluorescent probes or of other types of measurements is not consistent with the results. As expected, fluorescence polarization, ESR, and NMR [4] result in different values for the viscosity, because of the micelle intrinsic anisotropy, distinct interface properties, and different probe solubilization regions. Therefore, the method of intramolecular excimer emission can be applied to micellar solutions only for qualitative information regarding the variation of microviscosity due to modification of some environmental conditions.

Steady-state measurements of monomer and excimer fluorescence offer the advantage of simplicity; however, one has to take into account that some factors may have a direct bearing on the results. If there is a restricted solubilization of the probe, a fraction of the probe may remain in the solvent under the form of microcrystals, while in micelles of cationic surfactants with quaternary ammonium head groups, the aromatic probes are easily soluble due to the affinity for the head group [5,6], and in anionic surfactants micelles they have a longer solubilization time [7]. The freezing (at liquid nitrogen temperature) and thawing (at 23°C) of a SDS micellar solution

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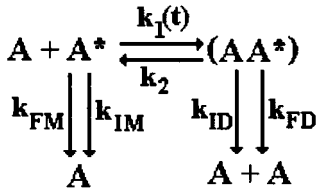
drastically accelerated the solubilization of the probe [8], dissolving the microcrystals.

Time-resolved measurements offer significant information concerning the kinetics of excimer formation and will indicate the relative importance of the dynamic process in the formation of intramolecular excimer. Moreover, the time difference between the maxima of the excimer fluorescence intensity and the exciting light pulse should be proportional to the viscosity.

The nature and the length of the chain that unites the two fluorescent molecules can influence the kinetics of the intramolecular excimer formation. It is generally considered that when the molecular chain which separates the two partners is long enough, and the solvent is an adequate one for the connecting chain, the kinetics can be controlled by the diffusion of the two fluorophors, just as in the case of intermolecular excimer. If this is not the case, the process may be non-diffusion-controlled, due to the existence of molecular conformers, that with a little rotation, a little modification of the relative positions of one fluorophore group with respect to the other leads to the intramolecular excimer formation.

The intermolecular (or intramolecular) excimer formation can be described by the following kinetic schema:



where $k_1(t)$ is the first-order rate constant for the excimer formation; k_2 is the rate constant for the excimer reversiblity; k_{IM} and k_{FM} are, respectively, radiationless and radiative decay rates of the excited monomer; and k_{ID} and k_{FD} are the corresponding rates for the excimer. If the rate constant for the excimer formation is considered to be independent of time, the exact solution of the differential equations resulting from the above kinetic schema, also called Birks' solution, ends up in a temporal evolution of the monomer and excimer concentrations represented by a sum of two exponential terms [9],

$$M = A_1\exp^{-\lambda_1 t} + A_2\exp^{-\lambda_2 t}$$
$$D = A(\exp^{-\lambda_1 t} - \exp^{-\lambda_2 t}) \quad (1)$$

However, because the excimer formation is diffusion-controlled, the rate constant k_1 must be time-dependent. Although several models were advanced, all assuming that the k_1 is given by Smoluckowki formalism, the results that represent the initial decay of the two fluorescent species, as well as the value of k_1, are not adequately modeled by any existing analytical expression, and the fitting of the experimental data is not possible [10–20]. Lee *et al.* [12] used a formalism based on a hierarchy of phenomenological kinetic equations for the reduced distribution function of reactant molecules. Agmon *et al.* [14] used modified boundary conditions in the evaluation of survival probabilities to induce reversibility. Following the principle of superposition of configurations, André *et al.* [11] have deduced the time evolution of monomer and excimer by an iterative procedure. Berberan–Santos *et al.* [17,18] make an explicit distinction between the excited monomer produced by light absorption and the excited monomer created by dissociation of a previous formed excimer, the geminate pairs, the two types of monomers having different distribution of the ground state. Martins *et al.* [20] consider the experimental fluorescence decay as the result of the superposition of the natural deexcitation and the result of the irreversible excimer formation reaction, while the law of the excimer fluorescence deexcitation is a convolution between deexcitation of the monomer and natural deexcitation of the excimer.

Several authors consider that the excimer formation constant decreases in inverse proportion to the viscosity in strongly viscous solvent [21]. However, the use of the short-chain bicromophoric molecules (e.g., dipyrenyl propane) as viscosity probes is questionable, because the rate constant cannot be directly correlated with the macroscopic viscosity of the solvent [22]. It was shown that the solvent microstructure that surrounds the probe exerts a greater influence in limiting of the motions of the two interacting groups than the bulk solvent viscosity. On the other hand, in a good solvent, the long spacer does not influence the diffusion of the fluorophors, but in a bad solvent for the chain, it is possible for the fluorophors to be very close to each other, almost parallel, inducing a nondiffusional formation of the excimer. The contribution of the geminate pairs (an excimer obtained by binding of a monomer and an excimer, originating from a dissociation of the previous excimer) to the total fluorescence is important in very viscous solvents [18].

Few theoretical models on the excimer formation in micelles are available. Some of them do not take into account the reversibility [23,24] (which is important only above 45°C [32]), while in the case of $k_2 \neq 0$, the master equation for the evolution of the concentrations of the fluorescent species is similar to that for homogeneous solutions, as long as the number of the fluorophore molecules per micelle is small and the monomers do not migrate between micelles and/or aqueous solution [33].

This contribution aims at investigating the variation of "microviscosity" in SDS micelles upon the addition

of BSA, making use of steady-state and time-resolved fluorescence of dipyrenyl decane measurements. Moreover, the purpose of the article is to evidence the effect upon the microviscosity of poly(oxyethylenic) polymer addition, with various degrees of polymerization, which interacts with SDS to a lesser or greater extent.

EXPERIMENTAL

Materials. Sodium dodecyl sulfate, especially pure (SDS; BDH), bovine serum albumin (BSA; Merck; 98% purity; 66,200-Da molecular mass), poly(ethylene oxide) (PEO; with 12,000, 600, and 200 molecular weights) and especially pure 1,10-di(1-pyrenyl)decane (DPD; Molecular Probes) were used without further purification. The fluorescent probe was added to the solutions as follows: a given amount of stock solution in cyclohexane was transferred to a volumetric flask and evaporated with a nitrogen stream; the micellar solutions were then added to it and magnetically stirred for 24 h. A 1×10^{-6} *M* concentration of DPD resulted. The solutions were degassed by nitrogen bubbling. To ensure equilibrium, the samples were measured after 3–4 days of room-temperature storage.

Steady-State Spectra. Steady-state spectra were recorded (at room temperature) on a SPEX Fluorolog 16 spectrofluorimeter, combined with SPEX DM3000 software. The slits were 1.5 (excitation) and 0.3 mm (emission). The excitation wavelength was 340 nm.

Time-Resolved Measurements. Time-resolved measurements were performed using the technique and experimental setup described in Ref. 25. All decay curves were deconvoluted from the lamp profile and response function of the detecting chain of apparatus recorded using a scatter solution (diluted fresh milk).

RESULTS AND DISCUSSION

Steady-State Fluorescence Measurements

In a previous paper [26], it has been shown that ionic surfactants aggregate as micelle-like clusters when an aqueous soluble protein is added in solution. The proposed model for SDS–BSA complex was of the "necklace and bead" type [24], protein chain wrapping partially around the micelle-like cluster of surfactants. The aggregation numbers obtained by steady-state and time-resolved fluorescence using pyrene solubilized in micellar aggregates of SDS in the presence of protein were smaller than those of free micelle [26]. At a protein concentration of 1%, and a mass ratio SDS/BSA of 0.50, the aggregation number was 39. The value increased with the mass ratio SDS/BSA so that at 1/1 the aggregation number was 78 [26]. Above this ratio, the values of aggregation number approached the corresponding value of free micelles.

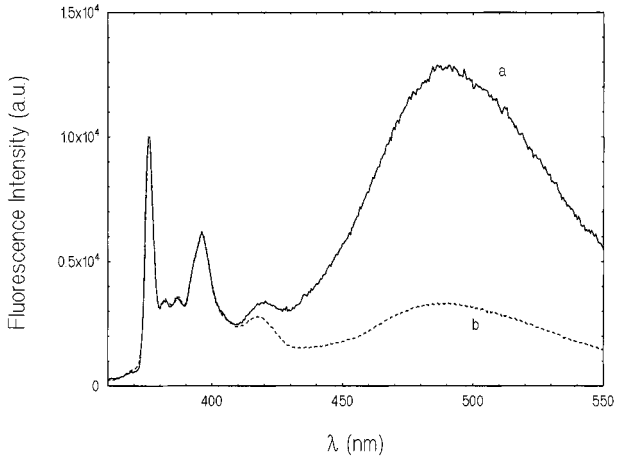


Fig. 1. Steady-state emission spectra of 1 μ*M* DPD in 1% SDS (curve a), and 1% SDS/0.75% BSA (curve b) solutions. The ionic strength was, in both cases, $I = 0.2$ *M*; $\lambda_{ex} = 340$ nm.

Figure 1 presents the fluorescence spectra of 1×10^{-6} *M* DPD in 1% SDS micellar solution and in 1% SDS–0.75% BSA solution. The effect of BSA addition is clearly a decrease in the excimer fluorescence band (with a maximum at 491 nm). This effect is a consequence of the microviscosity increase in micelle-like clusters formed by SDS in the presence of protein. The results of the I_E/I_M values measurements on this probe in 1% SDS micellar solutions in the presence of different protein concentrations are shown in Fig. 2. It is probable that the

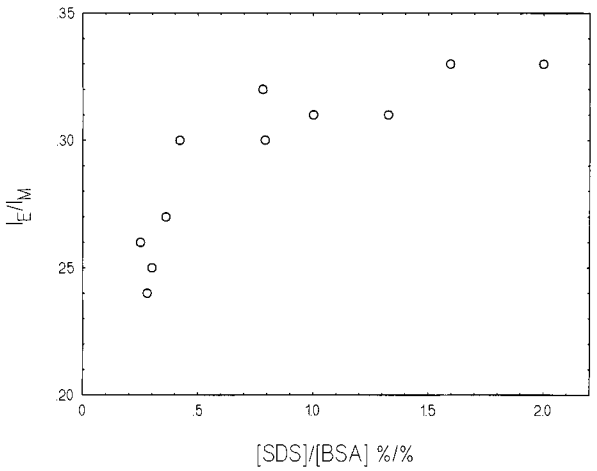


Fig. 2. The I_E/I_M values in 1% SDS solutions and different BSA concentrations.

microviscosity is higher in small micelle clusters than in free micelles, as previously concluded by Turro *et al.* from measurements on this system using pyrene as the fluorescent probe and 5-doxylstearic acid as the spin probe [24].

Figure 3 shows the important decrease in the I_E/I_M ratio, due to PEO addition to a 1% SDS solution (ionic strength $I = 0.2\ M$); the effect depends on the molecular weights of the added entities or, rather, on the polymer chain length and the strength of the polymer–surfactant interaction. In the case of the polymer with the highest molecular weight, the interaction is strong, the polymer chain is long, and even at a high ratio of SDS/PEO, clusters are still formed on the polymer chain. It is known that the aggregation number is lower in the presence of PEO then of free micelles and increases with the ratio SDS/PEO [27,28]. This increase in aggregation number is accompanied by a slight reduction in the microviscosity (one can note a slight tendency of the I_E/I_M ratio to increase). Cabane *et al*. [29] have studied the diluted SDS–PEO system, using small-angle neutron scattering experiments, and have proposed a model for the structure of the aggregated formed. They have observed that the behavior of the PEO with molecular weights higher than 10^4 is similar and that the PEO with lower weights do not associate with SDS. In Fig. 3 one can observe a decrease in the microviscosity in the case of polymers with lower molecular weights, but only at high polymer concentrations (low SDS/PEO ratio), where a weak SDS–PEO interaction is possible. The increase in the I_E/I_M ratio is fast for PEO with low molecular weights, because free micelles are formed. Since the chain is short, very few clusters are formed. The tendency is to approach the

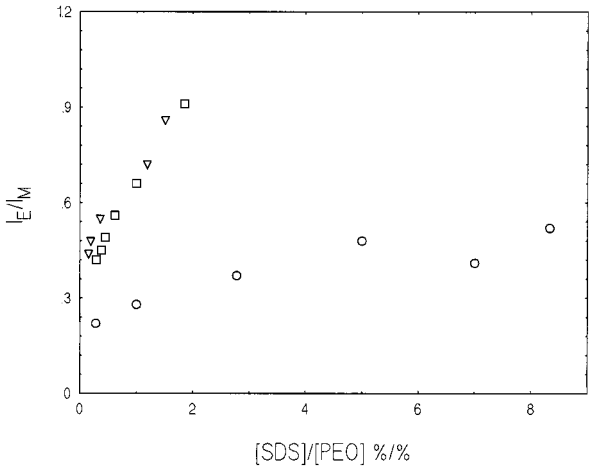


Fig. 3. The I_E/I_M values in different SDS/PEO systems, $I = 0.2\ M$: (∇) 1% SDS–PEO (MW = 200); (□) 1% SDS–PEO (MW = 200); (○) 1% SDS–PEO (MW = 12,000).

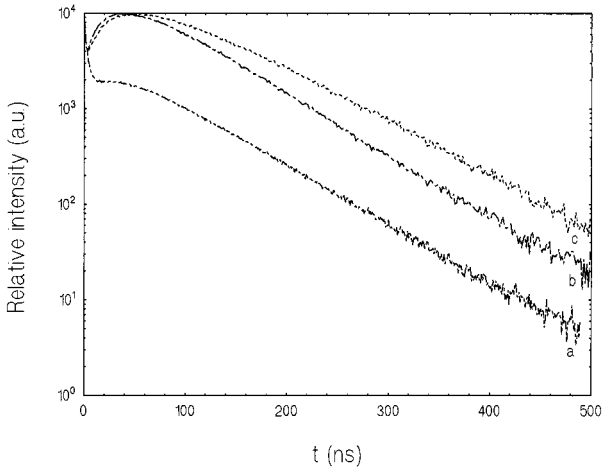


Fig. 4. The excimer fluorescence decay curves of DPD in PPO (a), hexadecane (b), and hexadecane + 50 % liquid paraffin (c).

I_E/I_M ratio in free micelles (1.28). At a low SDS/PEO ratio, i.e., below 0.5, the I_E/I_M value seems to be independent of the molecular weight of the polymer. At ratios higher then 0.5, the presence of free micelles in solution for the short-molecular chain polymers leads to a dependence of this ratio on the weight of the polymer.

Time-Resolved Fluorescence Measurements

Homogeneous Solutions. Figure 4 shows the excimer fluorescence decay curves of DPD in three solvents: hexadecane, hexadecane + 50% liquid paraffin, and polypropylene glycol (PPO; molecular weight, 2000). The fitting of both monomer and excimer experimental data to a sum of two exponentials proved to be a failure in all three cases (large χ^2 and nonsymmetrical distribution of the residuals). An additional exponential term leads to a better fit of the data in the case of both monomer and excimer fluorescence decays. Thus, the analytical shape advanced for the curves was

$$M^*(t) = A_1e^{-k_1t} + A_2e^{-k_2t} + A_3e^{-k_3t} \text{ and}$$
$$D^*(t) = A_4e^{-k_4t} + A_5e^{-k_5t} + A_6e^{-k_6t} \quad (2)$$

where k_i are rate constants. The attempt to fit the data with four exponentials does not improve the results (χ^2 remaining about the same).

Table I lists the parameters obtained following the fitting with three exponentials. In the case of the monomer, all amplitudes have positive values, while for the excimer one of them was negative. Birks' scheme is not valid, as the rate constant in the case of monomer and excimer should be the same, and the preexponential factors from evolution of the excimer should be equal, with

Table I. The Rate Constants (k_i; 10^6 s^{-1}) and the Preexponential Factors (A_i) Obtained from Fitting the Monomer and Excimer Fluorescence Decay Curves of DPD to a Sum of Three Exponentials [Eqs. (2)] in a Number of Deoxygenated Solvents and the Excimer Fluorescence Lifetime (τ_E; ns)

	Monomer		Excimer		
Solvent	k_1, k_2, k_3	A_1, A_2, A_3	k_4, k_5, k_6	A_4, A_5, A_6	τ_E
Hexadecane	3.050	0.165	15.384	0.490	
	22.609	0.507	35.026	−0.475	65.0
	125.313	0.326	323.624	0.033	
Hexadecane + 50% liquid paraffin	4.385	0.046	12.842	0.475	
	16.806	0.425	25.853	−0.452	70.5
	173.31	0.528	505.050	0.072	
PPO	9.315	0.318	9.615	0.307	
	39.016	0.092	20.900	−0.200	
	239.234	0.589	283.280	0.492	

opposite sign. In the case of hexadecane, the two amplitudes are around 0.5, but not identical, and a third exponential is required for good fit. Moreover, the rate constants are very different for the monomer and excimer decays.

In the case of PPO, the negative amplitude deviates considerably from 0.5, whereas the differences between the rate constants of the monomer and excimer are relatively small.

Because the solutions were degassed by nitrogen bubbling, it is possible that not all O_2 was removed from the solutions, so that in the kinetic schema in the Introduction, one could add the oxygen-quenching factor. However, this finding does not change the mathematics of differential equations in Birks' model. The presence of O_2 traces modifies only absolute values of the rate constants and not the relative shapes of the decay curves.

Some kinetic models, which lead to a good fitting with three exponentials, are presented in the literature. Snare *et al.* [30] considered two noninteracting conformers, having two different rate constants for excimer formation. However, in the kinetic scheme proposed, the monomer curve was fitted with a sum of two exponentials. Zachariasse *et al.* [31] found three exponential decays for the monomer as well as the excimer in the case of DPP (dipyrenyl propane) in toluene, ethanol, and cyclohexane, and the rate constants obtained have the same values for monomer and excimer decay curves. To explain this type of deexcitation, they suggested that there are two types of monomer and one excimer or one monomer and two excimers.

Berberan-Santos *et al.* [17,18] have shown that the contribution from excimers by reassociation of previously dissociated excimers is important in solutions with a high viscosity. Moreover, the model advanced by Berberan-Santos *et al.* shows that for an excimer in a given solution, depending on the diffusion coefficient, it is possible that monomer and excimer decay curves have different deexcitation rates even at long times. Unfortunately, the difficulty of obtaining the analytical form from Laplace inverse transformation makes the model intractable in data fitting.

The absence of a model that supplies analytical equations suitable for fitting prevents estimation of k_1, and implicitly of the diffusion coefficient. However, where the variation of the empirical fitting parameters with the viscosity in the homogeneous solutions is concerned, the rate constant corresponding to the exponential with negative amplitude from the excimer emission increases with viscosity. Thus, the addition of 50% liquid paraffin in hexadecane leads to a modification of the rate constant, with little change of the amplitudes. The amplitudes of the three excimeric exponentials behave differently. In hexadecane, the amplitude of the most rapid rate constant is low, but the amplitudes of the other two rate constants are very close to 0.5, the value expected for time-independent k_1. However, in PPO the amplitude of the fast rate constant becomes important, and in the case of monomer, in these three solvents, the amplitude of the fastest rate constant is important and increases with the viscosity. We assigned the fast kinetics to the contribution of geminate pairs at the deexcitation of the excimer, which became significantly in more viscous solutions. The correspondence in the rise time of "the geminate pairs" could not be evidenced because its kinetics is probably too rapid to be observed during the time window of the experiment (the time constant chosen for our experiments was 1.53 ns per channel).

The slowest rate constant of the monomer decay is close to the natural deexcitation rate constant of pyrenyl alkyl (e.g., $4.4 \times l0^6$ s^{-1} for octylpyrene in cyclohexane [30]). The lifetime of the excimer, τ_E (Table I), was evalu-

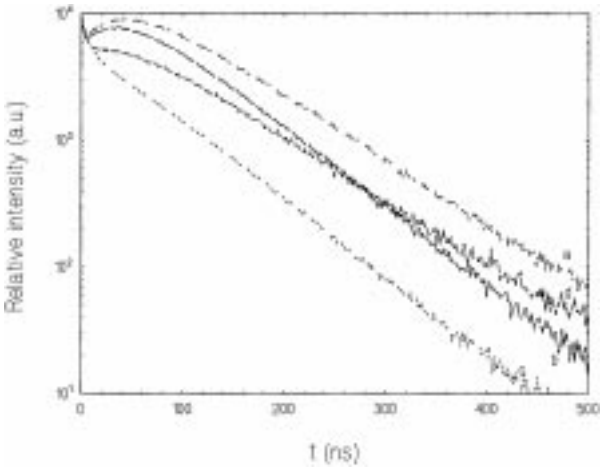


Fig. 5. The excimer fluorescence decay curves for different micellar solutions: 2% SDS, degassed and freeze–thaw (a); 2% SDS, nondegassed and freeze–thaw (b); 1% SDS, nondegassed (c); 1% SDS, degassed (d). $I = 0.2$ *M*.

ated making use of "the β-method" proposed by Duhamel *et al.* [33] The τ_E value in hexadecane is comparable with data available in the literature [30] for solutions of comparable viscosity ($\eta = 0.5$–1.2 cP), and in the other solutions, more viscous, the τ_E values are higher.

In Fig. 4 one can observe a shift in the maximum of the excimer decay curve toward longer times with increasing viscosity, as expected. On the other hand, one can note (Table I) that the rate constant corresponding to the exponential with negative amplitude, which is related to the formation of the excimer, increases with the solvent viscosity. Because in the case of viscous solution (PPO) the rapid kinetics becomes preponderant, and the clear estimation of the decay curve maximum is difficult, the rate constant with a negative amplitude can be used as an empirical parameter for estimation of the relative viscosity.

Microheterogeneous Solutions. The decay curves obtained in the case of SDS micellar solutions are shown in Fig. 5. It is known that pyrene and its derivatives are solubilized in micelles with the pyrenyl group toward the polar/hydrocarbonate interface. Because the chain which forms the micellar interior has the same chemical composition as the chain that connects the two pyrenyl groups of the probe, the chain will probably be solubilized in the interior of the micelle.

The fluorescence decay curves of the monomer have the same shape as the curve obtained in PPO. A good fit to the experimental data was obtained using a sum of three exponentials, for monomer as well as for excimer. The results are presented in Table II. Similarly, in the case of the solubilization of DPD in micelle-like clusters, the fitting with three exponentials proved to be the most convenient (Table III).

In SDS micellar solutions, the temporal evolution of the DPD excimer concentration is similar to that in PPO, which means that the amplitude of the fastest exponent term is high (0.225), although the other term with the negative amplitude exhibits a value approximately equal to that in hexadecane. An interesting finding is the difference in the shape of curves obtained by two methods of solubilization and degassing. By cycles of freezing–thawing, one can observe modifications of the fast and intermediate kinetics in the monomer curve and a decrease in the amplitude of the fast kinetics in the excimer decay curve (see Table II). This could lead to the conclusion that the conformers obtained in the two types of solubilization procedure differ. Thus, we consider that the probe molecule possesses a more extended conformation than in the case of solubilization by stirring, resulting in a smaller contribution of the geminate pairs to the excimer decay curve. The freezing process leads to a concentration of micelles and an acceleration of the probe solubilization, as was also observed by Kano [8]. Another significant observation is that the degassing of samples results in a decrease in the rate constant, whereas the amplitudes remain constant.

In the case of BSA–SDS solutions (Table III), one finds that the negative amplitudes are very low, and the highest amplitude, 0.7, is obtained for the largest rate constant. This behavior is also encountered for the monomer, where the amplitude is 0.8 for the fastest rate constant.

Comparing the rate constants with a negative amplitude for SDS with and without BSA, one finds that the smallest values are obtained in the free micelles. If the kinetics of excimer formation is the same as that in clusters, one can conclude that the microviscosity is slightly lower in the free micelles than in micelle-like clusters adsorbed on protein. However, the fact that the amplitudes of the fastest rate constant are important and much higher in clusters than in free micelles, suggests that the contribution of "the geminate pairs" have to be more important in the case of the clusters.

The value of the fastest kinetics seems to be the only parameter that is modified when the molar ratio SDS/BSA is varied. In this case, aerated samples have larger constants but unmodified amplitudes. Thus, in micelles and in clusters, although the presence of O_2 influences the excited state of the monomer and excimer by collisional quenching, the type of kinetics does not change, which suggests that the samples equilibrated with oxygen could be used, more easily, for obtaining dynamic fluorescence data.

Table II. The Rate Constants (k_i; 10^6 s^{-1}) and the Preexponential Factors (A_i) Obtained from Fitting the Monomer and Excimer Fluorescence Decay Curves of DPD to a Sum of Three Exponentials [Eqs. (2)] in SDS Solutions and the Excimer Fluorescence Lifetime (τ_E; ns)

	Monomer		Excimer		
Solution	k_1, k_2, k_3	A_1, A_2, A_3	k_4, k_5, k_6	A_4, A_5, A_6	τ_E
1% SDS, deoxygenated	4.523	0.102	11.456	0.473	
	16.490	0.427	34.952	−0.300	86.5
	224.71	0.469	167.22	0.225	
2% SDS, freeze–thaw, deoxygenated	4.295	0.128	11.875	0.489	
	13.704	0.698	29.656	−0.414	78.0
	177.46	0.173	184.160	0.095	
1% SDS, I = 0.2 M, oxygenated	7.219	0.116	14.50	0.516	
	17.161	0.651	30.30	−0.142	68
	69.060	0.231	118.90	0.341	
2% SDS, freeze–thaw, oxygenated	6.854	0.081	14.553	0.489	
	15.040	0.683	30.637	−0.400	63
	33.970	0.235	290.690	0.110	

The τ_E values in microheterogeneous solutions (Tables II and III) are higher than those found in homogeneous solutions, and moreover, excimer lifetimes seem to be longer when DPD is solubilized in micelle-like clusters in comparison with the case when it is solubilized in free micelles.

One has to underline that the fitting is obtained with a sum of three exponentials. The fact that the deexcitation constants for monomer and excimer are different could be assigned, leaving aside the diffusion process, to the recombination of pairs and possibly to the existence of another static process, which should lead to the formation of the excimer.

CONCLUSIONS

(1) The clusters formed by SDS on BSA protein possess a higher viscosity than free micelles.

(2) The model of the complex SDS–polymer, with a higher degree of polymerization, is similar to that proposed for SDS–BSA complex, their interaction leading to clusters with a higher microviscosity.

(3) In the case of polymers with a lower degree of polymerization, the interaction is very weak and happens only at low percentage ratios of SDS/polymer, in other words, at high polymer concentrations, when an increase in the microviscosity is noted.

ACKNOWLEDGMENTS

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Table III. The Rate Constants (k_i; 10^6 s^{-1}) and Preexponential Factors (A_i) Obtained from Fitting the Monomer and Excimer Fluorescence Decay Curves of DPD to a Sum of Three Exponentials [Eqs. (2)] in SDS/BSA Deoxygenated Solutions and the Excimer Fluorescence Lifetime (τ_E; ns)

	Monomer		Excimer		
Solution	k_1, k_2, k_3	A_1, A_2, A_3	k_4, k_5,k_6	A_4, A_5, A_6	τ_E
1% SDS + 0.5% BSA	5.208	0.063	9.354	0.257	
	14.285	0.107	26.860	−0.030	106.5
	282.48	0.828	204.08	0.712	
1% SDS + 1% BSA	5.740	0.082	9.330	0.153	
	16.173	0.113	26.852	−0.070	105.5
	258.13	0.803	347.220	0.775	
1% SDS + 0.5% BSA, oxygenated	8.333	0.163	10.790	0.306	
	20.165	0.168	28.694	−0.202	92.5
	301.20	0.667	532.12	0.491	

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