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Fluorescence lifetime distribution of 1,8-anilinonaphthalenesulfonate (ANS) in reversed micelles detected by frequency domain fluorometry

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Abstract

The fluorescence emission decay of ANS (1,8-anilinonaphthalenesulfonate) in reversed AOT (sodium bis-(2-ethyl-1-hexyl)sulfosuccinate) micelles at different water contents was investigated by frequency domain fluorometry. The whole ANS emission decay in reversed AOT micelles could not be fitted in terms of discrete lifetime values, i.e., mono-exponential and bi-exponential models. Better fits were obtained when using continuous unimodal Lorentzian lifetime distributions. This was interpreted as arising from the reorientation processes of water molecules around the excited state of ANS or probe exchange among different probe locations, occurring on a time scale longer than fluorophore lifetime. The dependence of ANS fluorescence anisotropy on the emission wavelength was consistent with te existence of a great emission heterogeneity especially for inverted micelles having reduced H_2O/AOT molar ratio. Finally, the observation that the distribution width decreases with increasing temperature and/or micelle size suggested that fast processes of water dipolar reorganization around the fluorophore are facilitated under these conditions.

Keywords: Reversed micelles; Fluorescence lifetime distribution; Frequency domain fluorometry; Anilinonaphthalenesulfonate emission decay

1. Introduction

Sodium bis-(2-ethyl-1-hexyl)sulfosuccinate (AOT) is a widely used surfactant forming reversed micelles in non-polar solvents. The polar head groups of the surfactant molecules constitute the core of the micelle, while the hydrophobic tails extend into the surrounding solution [1-5]. These systems may include aqueous solutions of biopolymers such as proteins, enzymes and genetic material [6-12]. The assembly of the water molecules entrapped in the polar core of reversed micelles is called "water pool" because of its unusual properties which differ from those of the bulk aqueous phase [13-16]. This observation may indicate that the "water pool" behaves as membrane-bound water mimetic system [3,17,18]. Since biopolymers solubilized in reversed AOT micelles show several unique fea-

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tures, the study of the characteristics of these systems is an useful way to understand the nature of the interactions responsible for important biological phenomena such as protein membrane interaction, folding, and enzyme action at the hydrophobic catalytic site [19–21].

Although theoretical and experimental studies provided a quite exhaustive picture of the thermodynamic and structural properties of the water molecule assembly entrapped in reversed micelles, the dynamic processes occurring in these systems on a time scale of micro and nanoseconds have not been clarified, yet [22,23]. A large variety of chemico-physical techniques has been employed to investigate the dynamic nature of water molecules in the inverted micelles such as ultrasonic and calorimetric investigations, as well as nuclear magnetic resonance, electron spin resonance, and raman spectroscopy, time domain and steady-state fluorescence [24–32]. From these investigations it was concluded that the dynamic nature of the water pool can be rationalized in terms of contributions associated with different types of water [24,29,30].

In this article, we have investigated the fluorescence emission decay of 1,8-anilinonaphthalene sulfonate (ANS) solubilized in reversed AOT micelles as function of temperature and water content. In particular, we used the multifrequency phase and modulation fluorometry, a technique that recently has been demonstrated to be very powerful in analyzing heterogeneous fluorescence emissions in proteins and membrane fragments [33–41]. Various function-models were considered in the data fit, i.e., discrete multiexponential and continuous lifetime distributions. The results show that the ANS emission decay in water containing reversed micelles is best fitted in terms of unimodal lifetime distributions having a Lorentzian shape.

2. Experimental

2.1 Materials

Sodium bis(2-ethyl-1-exyl)sulfosuccinate (AOT) and n-heptane, ethanol (HPLC grade), purchased from Sigma and Fluka respectively, were used without further purification. Sodium anilinonaphthalene sulfonate from Sigma was purified by repeated recrystallization from aqueous saturated $MgCl_2$ solutions. Double distilled water was used for preparing reversed micelles samples.

2.2 Micelle preparation

Water was solubilized in the AOT/n-heptane solution by stirring the mixture at room temperature for 20 min. The AOT concentration was maintained at 3.0% (w/v). Oxygen was removed by flushing with nitrogen.

2.3 Methods

Lifetime data were obtained by using a multifrequency cross-correlation phase and modulation fluorometer from ISS (La Spezia, Italy) ranging from 1 to 130 MHz, using a 300 W Xenon lamp as a light source. A solution of *p*-bis-(2-[5-phenyloxozolyl])benzene in ethanol served as lifetime reference [42]. The fluorescence was observed through a long-wave-pass filter with a cutoff wavelength at 400 nm (Corion LG-400-F) or interference filters with a full width at half maximum of 10 nm at the maximum wavelength transmittance (Corion 10P). The temperature of the sample compartment was controlled by using an external bath circulator. The sample temperature was measured prior to and after each measurement in the sample cuvette using a digital thermometer. The phase and modulation values were determined at 20 different modulation frequencies logarithmically separated from 1 to 130 MHz. The data were accumulated until standard deviations of phase and modulation were below 0.2° and 0.004, respectively. A non-linear least-squares program from I.S.S [43–46] was used for lifetime analysis, minimizing the chi-square function defined by:

$$\chi^2 = \sum_{\omega} \frac{1}{\sigma^2_{\phi\omega}} (\Phi_{\omega} - \Phi_{c\omega})^2 + \sum_{\omega} \frac{1}{\sigma^2_{m\omega}} (m_{\omega} - m_{c\omega})^2 \tag{1}$$

$$\chi^2_{red} = \frac{\chi^2}{2N - \gamma - 1} \tag{2}$$

Table 1

Lifetime analysis of ANS emission in reverse AOT micelles in n-heptane

W_0	Temperature (°C)	Discrete mono-exponential		Discrete bi-exponential				Continuous Lorentzian distribution		
		τ	χ^2	f_1	τ_1	τ_2	χ^2	C	W	χ^2
0.8	0	7.265	121.786	0.892	8.839	2.338	4.541	8.231	3.348	1.326
	10	6.260	130.286	0.879	7.725	2.078	3.035	7.075	3.223	1.312
	20	5.602	127.552	0.864	6.985	1.971	3.388	6.304	2.972	1.216
	30	5.201	115.734	0.869	6.407	1.808	3.031	5.820	2.685	1.016
	45	5.671	40.128	0.884	6.554	2.394	1.917	6.147	1.884	1.475
1.2	0	6.772	78.993	0.919	7.903	2.150	2.157	7.537	2.553	1.120
	10	7.160	16.985	0.839	8.245	4.024	1.577	7.594	1.978	1.063
	20	6.715	9.836	0.841	7.526	4.106	2.222	7.013	1.614	1.370
	30	6.306	3.599	0.561	7.397	5.268	1.657	6.454	1.124	1.408
3.2	0	6.336	53.395	0.903	7.321	2.322	1.650	6.944	2.204	1.260
	10	4.742	21.704	0.733	5.788	3.009	1.796	5.031	1.384	1.241
	20	4.120	2.646	0.956	4.278	2.019	0.991	4.201	0.709	1.061
8.2	0	3.924	1.953	0.385	4.689	3.541	1.317	3.977	0.567	1.190
	10	3.707	1.824	0.845	3.706	3.715	1.210	3.703	0.046	1.073
	20	3.097	2.122	0.472	3.192	3.017	1.278	3.099	0.087	1.390

AOT content was 3% (w/v). The excitation wavelength was 350 nm. The emission was observed through long pass wave filter (Corion LG 400 F).

where ω is the modulation frequency; σ_{Φ_ω} and σ_{m_ω} are the estimated standard deviations of measured phase angles and demodulations; Φ_ω and Φ_{c_ω} are the experimental and the calculated phase shifts; m_ω and m_{c_ω} are the experimental and the calculated demodulation factors, respectively; N is the number of measurementes per sample and γ is the number of free parameters.

The experimental data were analyzed assuming a sum of lifetime exponentials or a continuous distribution of lifetime values. Lifetime distributions are characterized by uniform (eq. 3), Gaussian (eq. 4), and Lorentzian (eq. 5) shape:

$$f(\tau) = \begin{cases} 1/W, & \text{for } C - W/2 \le \tau \le C + W/2 \\ 0, & \text{otherwise} \end{cases} \tag{3}$$

$$f(\tau) \propto \exp\left[-4 \ln 2\left(\frac{\tau - C}{W}\right)^2\right] \tag{4}$$

$$f(\tau) \propto \left[1 + 4\left(\frac{\tau - C}{W}\right)^2\right]^{-1} \tag{5}$$

The essential parameters of fitting are the center position (C) and the distribution width at half height (W).

Anisotropy measurements were performed on a Perkin Elmer MP-F66 spectrofluorometer. The anisotropy was calculated from:

$$A = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH})$$

where $G = I_{HV}/I_{HH}$; I is the fluorescence intensity, and the subscripts refer to the plane of polarization of the excitation and emission beams, i.e., V, vertical and H, horizontal emission.

3. Results

The fluorescence emission decay of ANS in reversed AOT micelles in n-heptane at different temperatures and water contents was measured by frequency domain fluorometry. The phase delays and demodulation factors observed by collecting the whole emission band through a long-wave pass filter were analyzed with the non-linear least-squares routines described in the Section 2. Different decay schemes, involving both discrete components and Lorentzian distributions of fluorescence lifetimes, were used. Table 1 summarizes the results.

The analysis, performed using the mono-exponential fit, although very poor, provided fluorescence lifetime values which appeared to be related to temperature and water content of reversed AOT micelles. More specifically, the lifetime decreases on increasing temperature and/or micelle size. The same dependence was found for the long lifetime component resulting from the bi-exponential fit. On the contrary, no meaningful correlation was observed between the value as well as the intensity fraction of the shortest lifetime component and temperature and/or micelle size.

A further improvement was obtained fitting the fluorescence decay data to continuous unimodal lifetime distributions of Lorentzian shape. Figure 1 shows the lifetime distributions of ANS in reversed micelles having different H_2O/AOT molar ratio (W_0), i.e., 0.8 and 8.2, at 0°C. The two distributions largely differ for both the position of

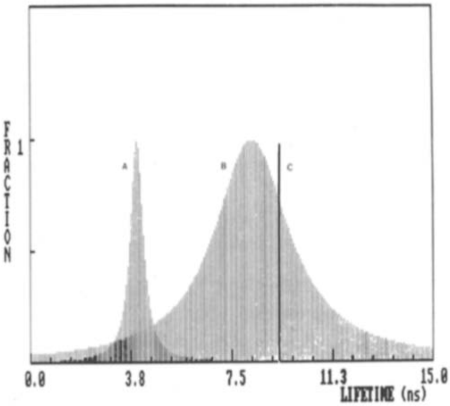

Fig. 1. Fluorescence lifetime distributions of ANS at 0°C in reversed micelles having different H_2O/AOT molar ratio: A, 8.2, B, 0.8. The samples contained 3% AOT (w/v). The vertical bar, C, indicates the lifetime value of ANS in ethanol, at the same temperature, obtained from the mono-exponential fit. Excitation was at 350 nm. Emission was observed through a LG 400 filter.

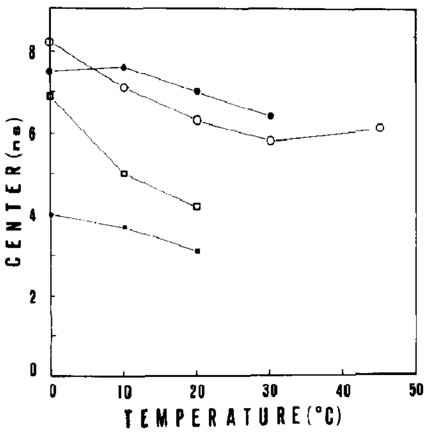

Fig. 2. Temperature dependence of the center of unimodal Lorentzian lifetime distribution of ANS solubilized in the water pool of reversed AOT micelles having different H_2O/AOT molar ratio: (○) 0.8, (●) 1.6, (□) 3.2, (■) 8.2. Excitation was at 350 nm. Emission was observed through a LG 400 filter. All samples contained 3.0% AOT (w/v).

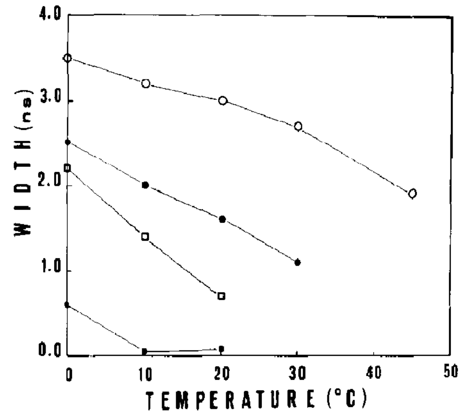

Fig. 3. Temperature dependence of the full width at half maximum of unimodal Lorentzian lifetime distribution of ANS solubilized in the water pool of reversed AOT micelles having different H_2O/AOT molar ratio: (○) 0.8, (●) 1.6, (□) 3.2, (■) 8.2. Other experimental conditions are reported in Fig. 2.

the center and full width at half maximum. In the case of micelles containing a low water content, the distribution center shifts towards a longer lifetime value and the distribution width increases. The same figure shows the single lifetime value that we found for ANS in non-viscous polar solvent, i.e., ethanol, at the same temperature. It is worthy of note that in the case of reversed AOT micelles in n-heptane in the absence of water ($W_0 = 0$) the decay data were best fitted in terms of a single lifetime, i.e., 6.4 ns.

Figures 2 and 3 show the dependence of the distribution center and full width at half maximum on water content and temperature. Again, faster emission decays were observed for the excited state of ANS molecules in reversed AOT micelles on increasing temperature and/or water content. Some other types of distributions were also considered (data not shown), i.e., Gaussian and uniform, but the Lorentzian distribution better fitted the experimental data as judged on the basis of the reduced chi-square values. However, although the application of the classical statistical *F*-test did not allow us to exclude these other distributional models for the Lorentzian one, the dependence of the distribution parameters obtained for the different models on temperature and H_2O/AOT molar ratio were similar.

Table 2 shows the dependence of the phase angles and demodulation factors measured at different frequencies on the emission wavelength for reversed AOT micelles for $W_0 = 2.0$ at 10°C.

Figure 4 shows the dependence of ANS fluorescence anisotropy in reversed micelles upon the emission wavelength. The anisotropy values were normalized to those observed at 560 nm. The decrease of anisotropy observed on increasing wavelength is very marked for reversed micelles having a reduced water content ($W_0 = 0.8$) if com-

Table 2

Phase shift angles and demodulation factors of ANS in AOT reversed micelles in n-heptane ($W_0 = 2$)

Frequency (MHz)		Emission wavelength (nm)			
		420	460	480	520
3.5	Phase	6.130	8.564	9.060	9.170
	Demod.	0.973	0.994	0.995	0.989
9.4	Phase	15.489	21.031	22.128	24.254
	Demod.	0.936	0.917	0.921	0.904
20.5	Phase	28.651	37.836	40.800	44.407
	Demod.	0.775	0.727	0.711	0.696
80.0	Phase	51.402	64.582	69.091	75.723
	Demod.	0.354	0.257	0.239	0.208

AOT content was 3% (w/v). The excitation wavelength was 350 nm. The emission was observed through interference filter (Corion 10P).

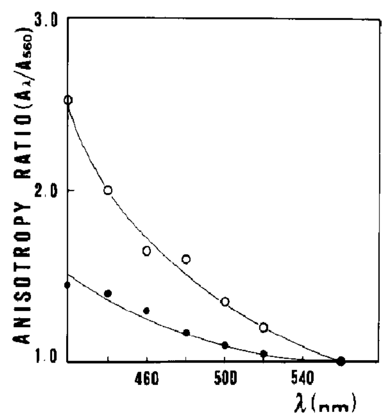

Fig. 4. Fluorescence anisotropy of ANS solubilized in the water pool of reversed AOT micelles as function of the emission wavelength at 15°C. Excitation was at 350 nm. All samples contained 3.0% AOT (w/v). (○) $W_0 = 0.8$, (●) $W_0 = 8.2$.

pared to the behaviour of the largest micelles ($W_0 = 8.2$). The actual values of anisotropy at 560 nm were 0.033 and 0.049 for the smallest ($W_0 = 0.8$) and largest ($W_0 = 8.2$) micelles, respectively.

4. Discussion

The behaviour of the aqueous core of reversed AOT micelles is different from that of bulk water, i.e., the core water shows restricted mobility and depressed freezing point. This can be attributed to the strong interaction of these water molecules with the ionic heads of the surfactant molecules, as well as to the partial distruption of the three-dimensional hydrogen-bonded network usually present in the bulk water. At least three types of water exist in the core of the droplets, i.e., ionic-head-bound water, bulk water and, for a small fraction, interface-trapped water [24,29, 30]. A fast exchange among the different types was suggested to occur on a nanosecond time scale or even lower [47]. The molecular organization and dynamics of the aqueous core of reversed AOT micelles have been investigated in the past by examining the steady-state fluorescence of ANS molecules included in the water cluster [48]. From these studies, it was concluded that the emission maximum characteristics of ANS fluorescence as well as the degree of fluorescence polarization are largely sensitive to the size of solubilized water pool. Moreover, several articles, performed following the emission decay of many other fluorescent probes, have shown the occurrence of excited state processes due to a very fast solvent dipolar reorientation around the fluorophore molecule [49–55]. The improvements made in the time resolved fluorescence techniques [56], specifically the new developments of the multifrequency phase shift and demodulation fluorometry, allowed us to reconsider the ANS emission decay in complex systems such as inverted AOT micelles with a reduced water content. It has been shown that the fluorescence decay of most anilinonaphthalensulfonates in non-viscous polar solvents can be satisfactorily described by a single lifetime [57]. Moore et al. reported a lifetime of 0.059 ns for ANS in pure water [57] using the 4th harmonic (263–264 nm) of a mode-locked Nd^{3+} phosphate glass laser for excitation. A single lifetime was found for 6-(*p*-toluidino)-2-naphthalenesulfonic acid (TNS) in pure ethanol [36]. The data reported in this paper indicate that the ANS emission decay in reversed AOT micelles of small size at low temperature can be interpreted by means of Lorentzian lifetime distribution except for micelles having $W_0 = 0$ which showed a mono-exponential decay. At higher temperatures and water contents, the statistical analysis does not allow to distinguish between the discrete exponential and continuous models (Table 1). However, the distribution model for the ANS decays seem to be the most realistic one also in these experimental conditions. In fact, the distributional parameters are related to the temperature and/or micelle size, whereas no meaningful or easy correlation can be found for the parameters obtained from discrete analysis.

The interpretation of the fluorescence decay of ANS incorporated in the water pool of reversed AOT micelles in terms of continuous lifetime distributions can be related to the emission heterogeneity generated by a multitude of environmental conditions. This is mainly due to the different orientations that the water dipoles, especially those forming the network bound to the ionic head of AOT, may assume around the excited fluorophore molecules or an exchange between different probe locations. This is further corroborated by the observation that in the absence of water the decay is represented by a single lifetime. The width of the lifetime distribution for water containing micelles depends on the number of orientations and locations, and on the interconversion rate among them. If the interconversion rate is slower than the fluorescence decay rate, the water network is virtually frozen for the duration of the excited state so that the emission arises from microenvironmental states which differ from each other. Obviously, this explanation does not exclude some other fast solvent dipolar reorganization during the excited state of ANS fluorophore [58–60] even in micelles with a reduced water content. The data shown in Table 2 are consistent with the occurrence of a solvent dipolar relaxation around the excited state of the fluorophore, i.e., the phase angles increase and the demodulation factor decrease on increasing emission wavelength.

The complex anisotropy dependence on emission wavelength, reported in Fig. 4, is also consistent with the above reported conclusion. In fact, the steady-state anisotropy A is related to rotational correlation time (τ_C) and fluorescence lifetime (τ_F) by Perrin equation:

$$A^{-1} = A_0^{-1}(1 + \tau_F/\tau_C)$$

where A_0 is the limiting anisotropy [61]. In a homogenous system having a single fluorescence lifetime, the anisotropy is constant on moving through the emission band so that the ratio A_λ/A_{red} equals unity. For a heterogeneous system, the observed anisotropy is the sum of each anisotropy component A_i:

$$A_\lambda = \Sigma f_i A_i$$

where f_i is the fraction for ith component having $\tau_{(F)i}$ as lifetime value. If the solvent dipolar reorientation is restricted, $\tau_{(F)}$ increases on going towards the red region of the emission band [58–60]. Therefore, a decrease of A_λ/A_{red} ratio is expected as function of the emission wavelength. In the case of reversed micelles having a very reduced content of water, a very marked dependence of A_λ/A_{red} on the emission wavelength was found (Fig. 4). This indicates that solvent dipolar relaxation is largely restricted. At higher values of water content, the less marked decreasing course of anisotropy indicates that a faster dipolar relaxation occurs. This behaviour can be ascribed to an increase mobility of water molecule in reversed micelles having a larger water content.

The decrease of the distribution center and width on increasing temperature and water content (Figs. 2 and 3) can also be related to a progressive enhancement of water fluidity that facilitates the solvent dipolar relaxation around the excited state of the fluorophore.

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