dye in the first monolayer. ϕ_{ET} is the two-dimensional energy transfer efficiency (BDA → DTC) in the same monolayer. (Some contribution of electron transfer may be included.) Contributions from direct excitation of DTC can be neglected, since the magnitude of the additional term due to this effect is estimated to be roughly

$$(\epsilon_{DTC}/\epsilon_{BDA})\eta_{DTC} \approx 0.3(0.1\eta_{BDA}) = 0.03\eta_{BDA} \quad (14)$$

The value of η decreases as a result of addition of DTC, because $\eta_{BDA} > \eta_{DTC}$. DTC thus acts as a quencher when it is incorporated in the same layer as BDA. Energy transfer from BDA to the a-dimer of BDA may occur to some extent. In this instance, however, this does not necessarily lead to reduction of photocurrent because the a-dimer of BDA may have some nonzero electron-injecting efficiency. Detailed results on monolayers containing BDA and DTC will be published elsewhere.[24]

In conclusion, we find a remarkable enhancement in the photocurrent with the multilayer system with BDA as an antenna dye and DTC as a reaction-center dye when BDA is incorporated in the outer layer and DTC is in the first layer in direct contact with a semiconductor electrode. The energy-transfer process can be explained in terms of the Förster model applied to the multilayer system.

Acknowledgment. We are grateful to Mr. Hirohisa Iwabayashi for his assistance in the experiments. This work was partially supported by a Grant-in-Aid for Scientific Research (60040004) of the Ministry of Education, Science, and Culture of Japan, and by the Asahi Glass Foundation for Industrial Technology.

Registry No. BDA, 41387-42-2; DTC, 26078-55-7; Sb, 7440-36-0; SnO_2, 18282-10-5; KCl, 7447-40-7; arachidic acid, 506-30-9; thiourea, 62-56-6.

Time-Resolved Fluorescence Depolarization of Rhodamine B and Octadecylrhodamine B in Triton X-100 Micelles and Aerosol OT Reversed Micelles

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(Received: April 2, 1987; In Final Form: August 7, 1987)

Time-resolved fluorescence and fluorescence anisotropy experiments were conducted on rhodamine B and octadecylrhodamine B incorporated into aqueous Triton X-100 micelles and in sodium bis(2-ethylhexyl) sulfosuccinate entrapped water or glycerol in a hydrocarbon solvent (heptane or dodecane). The time-resolved fluorescence behavior of the dye molecules in the micellar media was compared with that of the dye in homogeneous solution, from which a qualitative estimate of the polarity of the probe environment in the micelles could be inferred. The anisotropy decay of the fluorescent probes was analyzed with a biexponential decay model yielding correlation times characteristic for overall and internal micellar motion. The overall micellar rotation could be clearly distinguished from the faster internal motion in small water droplets and in glycerol droplets in heptane, for which there is good agreement between calculated and observed micellar rotation times. The hydrodynamic radii of glycerol droplets in dodecane medium are larger than the corresponding radii of droplets in heptane.

Introduction

Reversed micelles have been the subject of intensive interdisciplinary research during the past decade. Reviews on reversed micelles pertained to chemistry and biology,[1] biochemistry and enzymology,[2,3] biotechnology,[4] and spectroscopy.[2,5] Reversed micelles are usually considered as nanometer-scale, optically transparent water droplets, stabilized by a monolayer of surfactants in a bulk organic solvent. The most popular surfactant used thus far is sodium bis(2-ethylhexyl) sulfosuccinate (Aerosol OT or AOT), because no cosurfactant is required to form stable particles with a minimum interfacial tension between water and organic phase. A variety of techniques, summarized by Vos et al.,[5] have proven that reversed micelles are dynamic entities, which exhibit internal motion and communicate with each other on microsecond time scale. It has been recognized previously that time-resolved fluorescence techniques can be employed to investigate the (sub)nanosecond dynamics of reversed micelles.[6] In this investigation we describe the dynamic fluorescence properties of the versatile probe rhodamine B (RB) and the amphiphilic probe octadecylrhodamine B (ODRB) in AOT reversed micelles entrapped water or glycerol. Previously, ODRB has been utilized as a probe to monitor membrane fusion,[7] for distance determinations in biological aggregates,[8] and for the study of excitation transport in micelles.[9] Two organic solvents were used, namely, heptane and dodecane. The use of two different probe molecules enabled us to differentiate between two possible locations of the probe. We also varied the parameter w_0, which is defined as the molar ratio of surfactant and polar solvent (water or glycerol), in order to vary the size of the droplet. With photon correlation spectroscopy it was shown that the size of water-containing micelles is distinctly enhanced with increasing w_0.[10] The selection of different organic solvents is based on the different viscosities of heptane and dodecane. According to the Stokes–Einstein relation, variation of the viscosity would influence the rate of overall micellar rotation.

In order to interpret the time-resolved fluorescence in an appropriate manner, control experiments of the probes in well-defined media (solvents and normal micelles in water) are also described.

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0022-3654/88/2092-0759$01.50/0

The fluorescence lifetime of RB is very much dependent on the polarity and viscosity of the solvent and therefore is a good indicator of polarity and rigidity within the reversed micelle. Time-resolved fluorescence anisotropy measurements yield detailed information on the internal motion and overall micellar rotation. By judicious control of the experimental conditions we were able to separate both types of motion.

Experimental Section

Materials. Rhodamine B and rose bengal were obtained from Eastman Kodak. Octadecylrhodamine B chloride was purchased from Molecular Probes. Rose bengal was additionally purified to give a single spot on thin-layer chromatography as described earlier.[11] AOT was obtained from Fluka and purified further as earlier described.[12] Triton X-100 was from Merck (gas chromatographic grade). Most solvents were from Merck: heptane (analytical grade), methanol (fluorescent grade), and glycerol (fluorescence microscopy grade). Dodecane (99%) was obtained from Janssen Chimica. Heptane and dodecane were stored on molecular sieves and filtered prior to use. Water was purified on a Millipore system. In all the experiments the concentration of RB or ODRB was between 1.0 and 2.0 μM. Determinations were based on an extinction coefficient of 110 000 M^{-1} cm^{-1} in ethanol at 552 nm,[13] under the assumption that the extinction coefficient will not change much in the various systems.

Preparations of Reversed Micelles. Reversed micelles were prepared by addition of a predetermined volume of water, solvent, and aqueous RB solution to a 2-mL 66 mM AOT solution in the appropriate organic solvent until the desired w_0 (defined as the molar ratio of water and surfactant) was reached. The mixture was gently shaken until a clear solution was obtained. The end volume was 2.1 mL, which yielded a final, overall AOT concentration of 64 mM and a RB concentration of approximately 1.5 μM. ODRB was added as a preweighed solid to a reversed micellar solution. The largest operational w_0, before the onset of solution turbidity and phase separation, was about 17 for dodecane as organic phase. With heptane a w_0 as high as 50 can be used to obtain clear micelles. Reversed micelles containing glycerol were obtained by addition of 2.0-mL AOT solution in heptane or dodecane to a preweighed quantity of a RB or ODRB solution in glycerol, followed by mildly shaking for several minutes until a clear solution was obtained. The final concentration of AOT amounted to 66 mM. The highest w_0 (molar ratio of glycerol and AOT) was about 4.2 for heptane and approximately 1.0 for dodecane as hydrocarbon at 25 °C. Above these w_0 values the phase stability limit is exceeded, the solutions become turbid, and glycerol separates out. Reversed micelles were measured on the same day of preparation. All experiments with micelles were carried out at 25 °C.

Instrumentation. Absorption and steady-state fluorescence spectra were recorded on a Cary-16 spectrophotometer and an Aminco SPF-500 fluorimeter, respectively.

Time-resolved fluorescence data were recorded as described in detail elsewhere.[14] An argon ion laser (Coherent Radiation CR 18) was mode-locked and a rhodamine 6G dye laser (modified CR 590) was synchronously pumped to have 580-nm radiation as described earlier.[15] To decrease the 76-MHz repetition rate of exciting light pulses to 596 kHz, an electrooptic modulator setup was used.[16] The energy of exciting light pulses was in the subnanojoule region.

A Balzers B40 598-nm interference filter was combined with a RG 590 cutoff filter (Schott) to select fluorescence emission, and a fast 90° rotating sheet polarizer (Polaroid HN 22) was used to select emission polarized parallel or perpendicular with respect to the polarization direction of the excitation light.

Two different photon detectors were used in subsequent experiments: a Phillips PM 2254B photomultiplier and a Hamamatsu R 1645U-01 microchannel plate detector. The output of the latter tube was amplified by means of a preamplifier (Hewlett-Packard 8447 F), and an inverting transformer (EGG IT100) restored the negative amplitude to be used in the constant fraction discriminator (Canberra 1428 A).

With a time-to-amplitude converter (Ortec 457) the time distribution of fluorescence photons with respect to the excitation moment was converted into a series of analog pulses. The pulses were analyzed by using an ADC (Nuclear Data ND 570), and in two subgroups of the memory of a multichannel analyzer (Nuclear Data ND 66) data were collected of semisimultaneously recorded data of parallel and perpendicular polarized fluorescence. The data were transferred to a MicroVAX II computer for data analysis.

Data Analysis

Analysis of Polarized Fluorescence Decay. Full details of the data analysis, including the reference convolution method, have been given recently.[17] In this section we will restrict ourselves to models, where both the fluorescence, $s(t)$, and the anisotropy, $r(t)$, are composed of sums of exponential functions:

$$s(t) = i_{\parallel}(t) + 2i_{\perp}(t) = \sum_i \alpha_i \exp(-t/\tau_i) \quad (1)$$

$$r(t) = \frac{i_{\parallel}(t) - i_{\perp}(t)}{i_{\parallel}(t) + 2i_{\perp}(t)} = \sum_j \beta_j \exp(-t/\phi_j) \quad (2)$$

where $i_{\parallel}(t)$ and $i_{\perp}(t)$ are the parallel and perpendicular components of the fluorescence, respectively. The fluorescence lifetimes are denoted by τ_i, and the rotational correlation times by ϕ_j. The experimental quantities are $I_{\parallel}(t)$ and $I_{\perp}(t)$, which are convolutions of $i_{\parallel}(t)$ and $i_{\perp}(t)$ with the impulse response profile of the instrument $P(t)$. From $I_{\parallel}(t)$ and $I_{\perp}(t)$ the experimental, total fluorescence can be obtained from

$$S(t) = I_{\parallel}(t) + 2I_{\perp}(t) = P(t)\, s(t) \quad (3)$$

The impulse response profile $P(t)$ can be most accurately measured via the single-exponential fluorescence decay of the reference compound, $S_r(t)$, characterized by the lifetime, τ_r:

$$S_r(t) = P(t) \exp(-t/\tau_r) \quad (4)$$

The most widely used method to determine the decay parameters α_i and τ_i from $S(t)$ and $S_r(t)$ is the nonlinear least-squares method according to the following relationships:

$$S(t) = s(0)S_r(t) + S_r(t)\, X(t) \quad (5)$$

$$X(t) = \sum_i \alpha_i \left(\frac{1}{\tau_r} - \frac{1}{\tau_i} \right) \exp(-t/\tau_i) \quad (6)$$

In this procedure the weighted sum of the squared residuals, WSSR, is minimized:

$$\mathrm{WSSR} = \sum_{k=1}^{n} w_k [S(t_k) - S_c(t_k)]^2 \quad (7)$$

$S_c(t)$ is the calculated fit of $S(t)$, w_k is the weighting factor of data point $S(t_k)$, and n is the total number of data points. Since the noise for single photon counting data obeys Poissonian statistics, the weighting factors, which equal the inverse of the variance of the data points, can easily be calculated from the variances of $I_{\parallel}(t)$ and $I_{\perp}(t)$.[18] Minimization of WSSR can be performed with τ_r fixed, if an accurate value is known for it, or with τ_r as an extra iteration variable. A good method to obtain

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accurate values for the reference lifetime is to take two compounds, which decay single exponentially with clearly different lifetimes, and have the two decays fitted simultaneously with both lifetimes variable.

In this work we made use of rose bengal in methanol (or water) as reference compound. For rose bengal in methanol we obtained a lifetime of 530 ± 5 ps at 19 °C and 523 ± 5 ps at 25 °C, very close to the lifetimes reported previously.[19] For purified rose bengal in water the lifetime obtained was 66 ± 7 ps. In all these determinations RB in water served as single lifetime standard (see following section). A severe limitation of the reference method occurs when one of the lifetime values equals τ_r. In that particular case this component will not be found in the analysis because of elimination of the term $(1/\tau_r - 1/\tau_i)$ in eq 6.

Determination of the anisotropy parameters was carried out by a global analysis of $I_\parallel(t)$ and $I_\perp(t)$.[20] The basic equations are

$$I_\parallel(t) = P(t)\, i_\parallel(t) = P(t)\{\tfrac{1}{3}s(t) + \tfrac{2}{3}s(t)r(t)\} \quad (8a)$$

$$I_\perp(t) = P(t)\, i_\perp(t) = P(t)\{\tfrac{1}{3}s(t) - \tfrac{1}{3}s(t)r(t)\} \quad (8b)$$

$I_\parallel(t)$ and $I_\perp(t)$ can be fitted simultaneously with one set of parameters. The expressions for $I_\parallel(t)$ and $I_\perp(t)$ in case of a reference compound are obtained after substitution of eq 1 and 2 into eq 8a and 8b and Laplace and inverse transform of the resulting expressions, giving[17]

$$I_\parallel(t) = i_\parallel(0)S_r(t) + \tfrac{1}{3}S_r(t)\, X(t) + \tfrac{2}{3}S_r(t)\, Y(t) \quad (9a)$$

$$I_\perp(t) = i_\perp(0)S_r(t) + \tfrac{1}{3}S_r(t)\, X(t) - \tfrac{1}{3}S_r(t)\, Y(t) \quad (9b)$$

$$Y(t) = \sum_i \sum_j \left\{ \alpha_i \beta_j \left(\frac{1}{\tau_r} - \frac{1}{\tau_i} - \frac{1}{\phi_j} \right) \exp\left(-\frac{t}{\tau_i} - \frac{t}{\phi_j} \right) \right\} \quad (10)$$

In the derivation of these equations it is assumed that when $s(t)$ and $r(t)$ are multiple exponential, all the cross terms for the different lifetimes τ_i and correlation times ϕ_j must be accounted for. Physically, this means that every lifetime component is coupled with every rotation. For instance, in the case of a mixture of two different fluorescent compounds with each a single lifetime and correlation time, these lifetimes must be only associated with one of the correlation times. Hence, the model has to be adapted and some of the cross terms have to be omitted. In this investigation it is assumed that every lifetime is associated with every rotation, since a single chromaphoric group is considered.

The quality of the fit is judged with visual inspection and with the help of various statistical functions and parameters, namely, the weighted residuals, the autocorrelation function of the weighted residuals, the number of sign changes in the autocorrelation function,[21] the reduced χ^2 value, and the Durbin–Watson parameter (DW).[22] The standard deviations of the fitted parameters can be estimated from the Hessian,[23] which is one of the output parameters of the computer program.

Models in Fluorescence and Anisotropy Decay. For the probes in (reversed) micelles the fluorescence decay turned out to be nonexponential and could be fitted with a sum of two exponential functions:

$$S(t) = \alpha_1 \exp(-t/\tau_1) + \alpha_2 \exp(-t/\tau_2) \quad (11)$$

Lifetime heterogeneity was interpreted to arise from the same fluorophore but located in a nonhomogeneous environment. Analysis of the fluorescence decay into two exponential functions is probably a simplification but has the advantage of a more simple, qualitative interpretation. If it is assumed that the probe is located at the surfactant boundary layer between entrapped solvent and oil phase, such a decay model is a fair approximation, since the probe faces two distinct environments.

Anisotropy decay data of the probes in (reversed) micelles could be adequately fitted to a biexponential decay:

$$r(t) = \beta_1 \exp(-t/\phi_1) + \beta_2 \exp(-t/\phi_2) \quad (12)$$

with $\phi_1 < \phi_2$ and $\beta_1 + \beta_2 = r(0)$, the initial anisotropy. The correlation time ϕ_2 is characteristic for the overall, micellar rotation, and ϕ_1 is a time constant reflecting internal motion of the probe. The internal motion of the probe can be analyzed with a wobbling-in-cone model,[24] in which it is assumed that the emission transition moment coincides with the axis, that wobbles in a cone of semiangle θ_c. In the latter model ϕ_1 is a composite correlation time: $1/\phi_1 = 1/\phi_2 + 1/\phi_{int}$, where $1/\phi_{int}$ is related to the wobbling diffusion coefficient. From the amplitude of the exponential describing micellar rotation, β_2, the cone angle can be obtained from

$$\beta_2/r(0) = \left[\frac{1}{2} \cos\theta_c (1 + \cos\theta_c) \right]^2 \quad (13)$$

On the other hand, the overall, micellar rotational correlation time, ϕ_{mic}, can be evaluated from the Stokes–Einstein relation for spherical particles:

$$\phi_{mic} = \frac{1}{6D_{rot}} = \frac{4\pi r_h^3 \eta}{3kT} \quad (14)$$

D_{rot} is the rotational diffusion coefficient, η the viscosity, k the Boltzmann constant, T the absolute temperature, and r_h the hydrodynamic radius. The hydrodynamic radii of AOT reversed micelles containing either water or glycerol with heptane as oil phase were evaluated from photon correlation spectroscopy, leading to the following empirical relationships:

for water droplets:[25]

$$r_h = 1.5 + 0.175w_0 \quad (15)$$

for glycerol droplets:[26]

$$r_h = 1.7 + 0.88w_0 \quad (16)$$

The solvent viscosities used were 0.386 cP (25 °C) for heptane and 1.35 cP (25 °C) for dodecane.[27]

Results and Discussion

Fluorescence Dynamics in Different Solvents and Triton X-100 Micelles. As also concluded earlier, RB in water can be considered as a single fluorescence lifetime standard.[28] This was confirmed by our dual fluorescence lifetime measurements using rose bengal in methanol as reference compound (Figure 1). We found a lifetime of 1.50 ns at 25 °C. It was noted, however, that the fluorescence lifetime becomes longer at lower temperature; for instance, at 19 °C we found 1.77 ns. The value of 1.63 ns reported by Kemnitz et al.[28] (at unknown temperature) is right between these values. All data are collected in Table I. The lifetime is

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TABLE I: Fluorescence Lifetimes and Rotational Correlation Times of Rhodamine B and Octadecylrhodamine B in Water, Methanol, Glycerol, Heptane, Dodecane, and Triton X-100 Micelles[a]

system	α_i	τ_i, ns	β_j, ns	ϕ_j, ns
RB/W	1.0	1.50 ± 0.02	0.38[b]	0.18[c]
RB/W (19 °C)	1.0	1.775 ± 0.005		
RB/M	1.0	2.28	0.35	0.12[c]
RB/G	−0.093 ± 0.007	0.41 ± 0.04	0.376 ± 0.001	58 ± 8
	1.093 ± 0.002	3.400 ± 0.005		
RB/G (19 °C)	−0.091 ± 0.007	0.45 ± 0.04	0.374 ± 0.001	81 ± 3
	1.091 ± 0.002	3.504 ± 0.004		
ODRB/M	1.0	2.25	0.35	0.12[c]
ODRB/H	0.57	0.16		
	0.33	0.33		
	0.10	3.80		
ODRB/D	0.21	0.71		
	0.62	1.60		
	0.17	3.72		
ODRB/T	0.13 ± 0.01	1.56 ± 0.09	0.11 ± 0.01	2.0 ± 0.2
	0.87 ± 0.01	2.84 ± 0.01	0.25 ± 0.01	8.8 ± 0.4
ODRB/T[d]	0.15 ± 0.03	1.79 ± 0.11	0.11 ± 0.01	2.0 ± 0.2
	0.85 ± 0.03	2.78 ± 0.02	0.25 ± 0.01	10.2 ± 0.5

[a] All measurements at 25 °C, unless otherwise indicated. Abbreviations: RB = rhodamine B; ODRB = octadecylrhodamine B; W = water; M = methanol; G = glycerol; H = heptane; D = dodecane; T = Triton X-100. [b] Only for RB in water at 25 °C is the standard deviation based on five separate experiments; all other standard deviations refer to the fit. [c] Rapid, predominant, component of biexponential decay. [d] Duplicate experiment.

more than doubled in glycerol as solvent (Table I). In the latter analysis a buildup of fluorescent species was evident from a small negative preexponential factor and short time constant. Since we had to detect the red portion of the emission because of the red edge excitation employed, the rise of the fluorescence in glycerol can be interpreted by retarded solvent relaxation around the excited chromophore. In a more fluid solvent like methanol the fluorescence decay is exponential with a lifetime of 2.3 ns for both RB and ODRB (Table I).

In Table I the experimental limits are given for the time-dependent anisotropies of RB in water and glycerol, respectively. Within the fluorescence observation range of 30 ns the fluorescence anisotropy of RB in glycerol is exponential with a rotational correlation time of 58 ns. The rotational correlation time follows the viscosity of the solvent since a longer time constant is observed at slightly lower temperature (80 ns at 19 °C, Table I). The rotational correlation time of RB in water was found to be 180 ps at 25 °C. In the latter case the initial, apparent anisotropy is lower than the true one because of the finite pulse width, as has been shown by simulations.[29]

Fluorescence decays of ODRB in heptane or dodecane were found to be highly nonexponential (Table I). The latter results can be explained by the formation of aggregates of similar nature as has been found for aggregated RB species adsorbed on fused quartz plates.[28] The same long lifetime component as found here lends strong credence to this explanation. ODRB is, owing to its long hydrocarbon chain, very soluble in Triton X-100 micelles in water.[9] We have selected this system for two obvious reasons. Aqueous micelles are dynamic entities in which water can penetrate to a certain extent. The polar RB moiety can be expected to be positioned in the outer, micellar layer in the vicinity of the aqueous solvent. In addition, because of the large fluidity of the micellar interior, internal motion superimposed on the micellar rotation must become evident in the fluorescence anisotropy decay. Both expectations were indeed corroborated by the experiments. The fluorescence decay of ODRB in Triton X-100 micelles is biexponential (Table I) with a contribution of a lifetime component of 1.6 ns which forms evidence for a partial contact of the probe with water. The predominant component is longer and reflects the more apolar environment. A monoexponential decay model does not hold in this system as judged from the various criteria as presented in the Data Analysis section.

The decay of the fluorescence anisotropy of ODRB in Triton X-100 micelles is nonexponential as well as observed in Figure 2. As pointed out in the preceding section, it is assumed that both fluorescence lifetime components are associated with the same

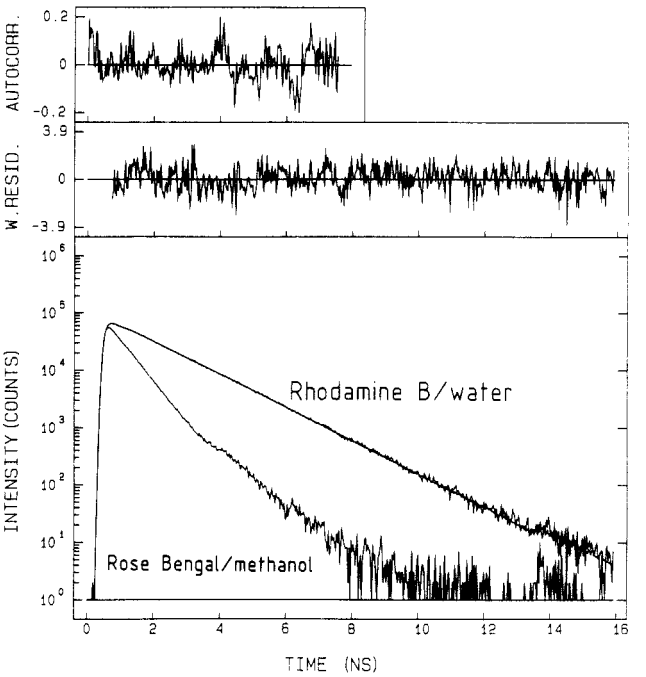

Figure 1. Example of fluorescence decay analysis of rhodamine B (RB) in water with rose bengal in methanol as reference compound at 25 °C. Shown are three response curves, namely, the experimental fluorescence of RB and the calculated response with a lifetime of 1.500 ± 0.002 ns. The fluorescence lifetime of rose bengal in methanol was simultaneously fitted to 0.523 ± 0.002 ns. On top the weighted residuals between calculated and experimental fluorescence decay and the autocorrelation function of the residuals (first point omitted) are shown. Fit criteria for this fit are χ^2 = 1.05, Durbin–Watson parameter DW = 1.66, and number of zero passages in the autocorrelation function ZP = 99; number of channels used is 500; time equivalence per channel is 31 ps. The microchannel plate detector was used.

probe motion (homogeneous rotation). Two correlation times were found: a shorter one of around 2 ns and a longer one of about 9 ns. The longer correlation time reflects the rotation time of the whole micelle. The shorter correlation time must be ascribed to rapid restricted motion of the probe within the micelle. It is possible to estimate the angular displacement (θ_c) using the formalism given in the preceding section. The amplitude β_2 belonging to the longer correlation time is related to θ_c via eq 13. The angular displacement of the rhodamine ring amounted to 28°. Similar results as described here were obtained from a parinaroylphosphatidylcholine lipid molecule in Triton X-100 micelles.[30]

(29) Papenhuijzen, J.; Visser, A. J. W. G. *Biophys. Chem.* **1983**, *17*, 57.

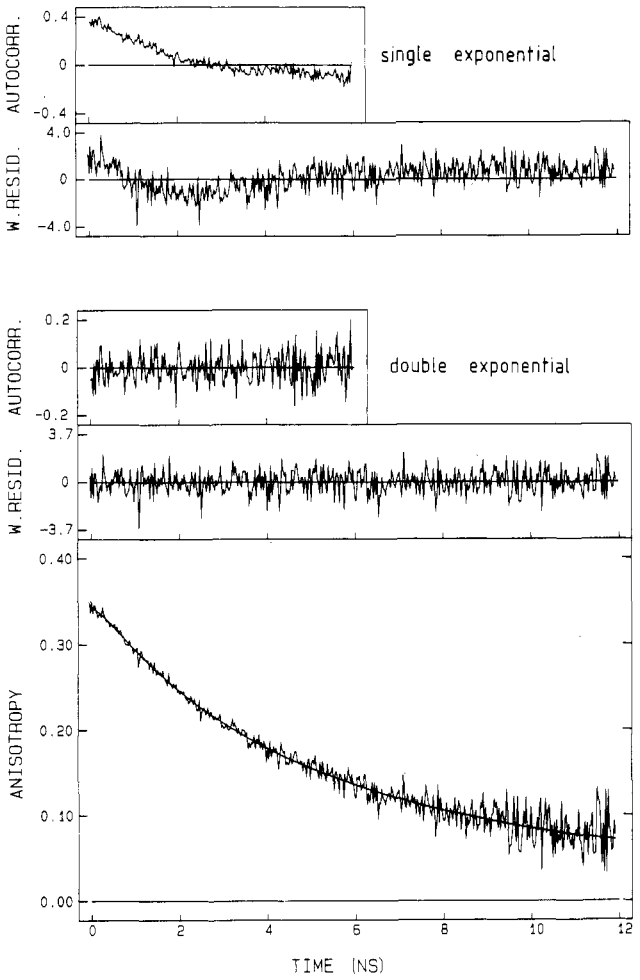

Figure 2. Anisotropy decay analysis of octadecylrhodamine B (ODRB) in Triton X-100 micelles in water at 25 °C. Analysis started 10 channels before occurrence of the peak intensity of the reference compound and was carried out over the whole fluorescence decay profile (1024 channels). Only the initial anisotropy decay is presented. The parameters of the biexponential decay are listed in Table I (first of two experiments). Fit criteria are $\chi^2 = 1.03$, DW = 1.97, and ZP($I_\parallel$) = 242 and ZP($I_\perp$) = 245. The upper panel presents the residuals and the autocorrelation function for a single-exponential fit (ϕ = 6.0 ns). Based on the lesser quality of this fit ($\chi^2 = 1.21$, DW = 1.68, ZP($I_\parallel$) = 176 and ZP($I_\perp$) = 200), a single-exponential decay model is not adequate.

In the latter case of a different probe molecule a biexponential anisotropy decay was obtained as well at 20 °C, with correlation times of 2.1 and 7.8 ns, respectively, and angular displacement of 32°.

Fluorescence Dynamics in AOT Reversed Micelles Entrapped Water in Heptane and Dodecane. The fluorescence decay was in all reversed micelles complex, and biexponential functions were needed to adequately fit the experiments. All results are collected in Table II. Interestingly, the component associated with the aqueous environment shows up in micelles containing ODRB. In the smallest micelles with the lowest water content the predominant component is a long lifetime of similar value as found for RB in glycerol. Especially in case of RB as probe in small micelles contact of the probe with the outer organic solvent is strongly suggested. It is known that in small micelles all water molecules are used up in hydrating the polar head groups.[31] At increasing water content the difference between the two lifetimes disappears and the average lifetime gets distinctly shorter, indicating that the probe gets surrounded by "normal" water molecules. The latter behavior is independent of the nature of the organic solvent.

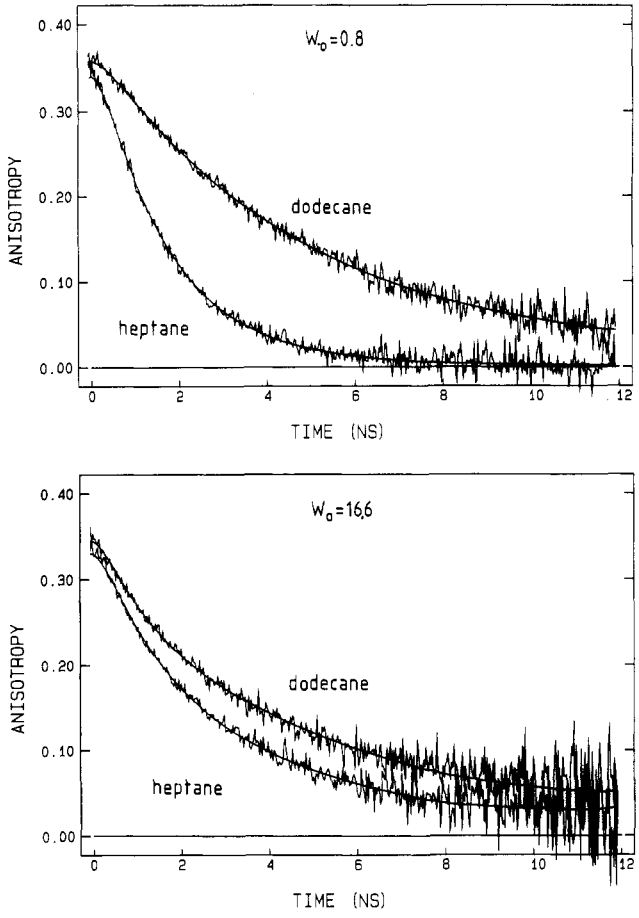

Figure 3. Anisotropy decay profiles and their fits of RB in AOT reversed micelles entrapped water in heptane and dodecane. The fits are for biexponential functions with parameters listed in Table II. The upper panel is for w_0 = 0.8, and the lower panel for w_0 = 16.6. Note the more rapid decay of the droplet in dodecane at w_0 = 16.6 as compared to w_0 = 0.8.

If the lifetimes of the two probes in small micelles (w_0 = 0.8) are compared, it is clear that ODRB senses a partly aqueous environment, whereas RB is in a rather apolar medium. At higher w_0, when the reversed micelle grows and the surfactant number increases, the difference between RB and ODRB fluorescence decay parameters gets smaller. The results obtained with small micelles can be explained by assuming that the packing of the surfactants is perturbed by the hydrocarbon chain of ODRB. The long aliphatic chain has strong preference for the apolar regions of the micellar system, while the charged chromophore tends to reside near the water. At the largest micelles (w_0 = 16.6) the fluorescence decay characteristics of the two probes become identical, a conclusion also reached by Rodgers[32] using rose bengal derivatives as probes. The shorter fluorescence lifetime, however, of both probes never approaches the one of RB in water (1.5 ns), which is an indication that the probe remains bound at the interface, probably through electrostatic interaction. Such a conclusion was also obtained previously from similar experiments with fluorescein as probe in reversed micelles of AOT in isooctane.[6a]

We prefer to present the two-component analysis in order to establish the trends in the changes of parameter values. The average lifetime characterizes the overall decay behavior.

Based on the fitting criteria as given in the Data Analysis section and illustrated for ODRB in Triton X-100 (Figure 2), the fluorescence anisotropy decay is biexponential in all cases. The shorter correlation time reflects a restricted motion of the probe within the micelle. The longer correlation time contains a contribution of the rotation of the whole micelle. In Figure 3 two comparative examples are shown for reversed micelles at high (w_0

(30) Visser, A. J. W. G.; Van Hoek, A.; Van Paridon, P. A. In *Membrane Receptors, Dynamics and Energetics*; Wirtz, K. W. A., Ed.; Plenum: New York, 1987.

(31) Wong, M.; Thomas, J. K.; Nowak, T. *J. Am. Chem. Soc.* **1977**, *99*, 4730.

(32) Rodgers, M. A. J. In *Reverse Micelles*; Luisi, P. L., Straub, B. E., Eds.; Plenum: New York, 1984.

TABLE II: Fluorescence Lifetimes and Rotational Correlation Times of Rhodamine B and Octadecylrhodamine B in AOT Reversed Micelles Entrapped Water in Heptane or Dodecane at 25 °C[a]

system	w_0	α_i	τ_i, ns	$\bar{\tau}$,[a] ns	β_j	ϕ_j, ns
RB/H	0.8	0.26 ± 0.04	2.8 ± 0.1	3.7	0.16 ± 0.09	1.0 ± 0.2
		0.74 ± 0.04	3.99 ± 0.04		0.21 ± 0.07	1.9 ± 0.2
RB/H	5.0	0.49 ± 0.10	2.24 ± 0.04	2.4	0.14 ± 0.02	1.1 ± 0.1
		0.51 ± 0.10	2.61 ± 0.04		0.22 ± 0.02	3.4 ± 0.2
RB/H	16.6	0.48 ± 0.11	2.00 ± 0.03	2.1	0.13 ± 0.01	0.94 ± 0.06
		0.52 ± 0.11	2.13 ± 0.03		0.22 ± 0.01	4.3 ± 0.1
RB/D	0.8	0.31 ± 0.07	2.8 ± 0.1	3.5	0.15 ± 0.05	3.2 ± 0.4
		0.69 ± 0.07	3.76 ± 0.05		0.22 ± 0.05	6.3 ± 0.5
RB/D	5.0	0.37 ± 0.12	2.4 ± 0.1	2.7	0.08 ± 0.01	1.6 ± 0.2
		0.63 ± 0.12	2.91 ± 0.08		0.28 ± 0.01	7.5 ± 0.2
RB/D	16.6	0.23 ± 0.08	1.81 ± 0.07	2.1	0.096 ± 0.004	0.73 ± 0.05
		0.77 ± 0.08	2.20 ± 0.02		0.268 ± 0.004	5.7 ± 0.1
ODRB/H	0.8	0.33 ± 0.01	1.74 ± 0.03	2.9	0.16 ± 0.04	1.0 ± 0.1
		0.67 ± 0.01	3.48 ± 0.01		0.21 ± 0.04	2.2 ± 0.2
ODRB/H	5.0	0.48 ± 0.08	1.97 ± 0.04	2.2	0.10 ± 0.01	0.72 ± 0.06
		0.52 ± 0.08	2.39 ± 0.03		0.26 ± 0.01	2.88 ± 0.07
ODRB/H	16.6	0.64 ± 0.05	1.92 ± 0.01	2.0	0.14 ± 0.01	0.78 ± 0.05
		0.36 ± 0.05	2.15 ± 0.02		0.21 ± 0.01	4.0 ± 0.1
ODRB/D	0.8	0.25 ± 0.01	1.68 ± 0.04	2.9	0.13 ± 0.03	2.7 ± 0.3
		0.75 ± 0.01	3.37 ± 0.01		0.23 ± 0.03	7.1 ± 0.5
ODRB/D	5.0	0.44 ± 0.08	2.07 ± 0.07	2.4	0.10 ± 0.01	1.4 ± 0.1
		0.56 ± 0.08	2.72 ± 0.05		0.26 ± 0.01	7.5 ± 0.3
ODRB/D	16.6	0.60 ± 0.07	1.95 ± 0.02	2.1	0.114 ± 0.005	0.95 ± 0.06
		0.40 ± 0.07	2.22 ± 0.03		0.237 ± 0.006	6.2 ± 0.2

[a] See Table I for abbreviations and details. [b] Average lifetime $\bar{\tau} = \alpha_1\tau_1 + \alpha_2\tau_2$.

TABLE III: Fluorescence Lifetimes and Rotational Correlation Times of Rhodamine Band Octadecylrhodamine B in AOT Reversed Micelles Entrapped Glycerol in Heptane or Dodecane at 25 °C[a]

system	w_0	τ, ns	β_j	ϕ_j, ns
RB/H	0.6	3.73 ± 0.02	0.17 ± 0.04	2.2 ± 0.3
			0.20 ± 0.04	5.4 ± 0.5
RB/H	3.4	3.714 ± 0.003	0.063 ± 0.004	5.4 ± 0.7
			0.311 ± 0.004	50 ± 6
ODRB/H	0.95	3.70 ± 0.01	0.13 ± 0.02	2.0 ± 0.2
			0.24 ± 0.02	6.5 ± 0.4
ODRB/H	4.1	3.597 ± 0.003	0.08 ± 0.01	5.1 ± 0.8
			0.29 ± 0.01	59 ± 9
RB/D	0.6	3.670 ± 0.003	0.13 ± 0.03	4.9 ± 0.7
			0.24 ± 0.03	23 ± 3
RB/D	1.2	3.632 ± 0.003	0.12 ± 0.02	6.2 ± 0.6
			0.25 ± 0.02	49 ± 8
ODRB/D	0.7	3.534 ± 0.003	0.16 ± 0.02	5.7 ± 0.6
			0.21 ± 0.02	31 ± 4

[a] See Table I for abbreviations and details.

= 16.6) and low (w_0 = 0.8) water content. The rotational correlation time of the whole micelle is, however, expected to be much longer for larger micelles (see next section). An increase in viscosity of the organic solvent by a factor of 3.5 by taking dodecane instead of heptane would affect the rotational correlation time by the same amount. Such an increase is only observed at the lowest w_0, e.g., at w_0 = 0.8 ϕ_2 = 1.9 ns in heptane and ϕ_2 = 6.3 ns in dodecane for RB and, similarly, ϕ_2 = 2.2 and 7.1 ns for ODRB. At w_0 = 16.6 the longer corelation time is even shorter than at w_0 = 5.0 for both probes. The latter observation forms evidence that time constants of internal motion are also appearing in the longer correlation time. Comparing the two solvents, it is clear that at w_0 = 0.8 and, to a lesser extent, w_0 = 5.0 the shorter correlation time becomes longer in the more viscous solvent. The latter observation is another line of evidence that the organic solvent penetrates the interfacial layer, so that the probe experiences the increased viscosity arising from dodecane. At w_0 = 16.6 the short correlation time is very similar in both external organic solvents.

Fluorescence Dynamics in AOT Reversed Micelles Entrapped Glycerol in Heptane and Dodecane. The recent work of Fletcher et al.[26,33] with photon correlation spectroscopy and quasi-elastic neutron scattering on AOT reversed micelles with glycerol entrapped stimulated us to use glycerol instead of water. Since the viscosity of glycerol is approximately 1500 times larger than that of normal water, it is likely that the internal motions can be frozen

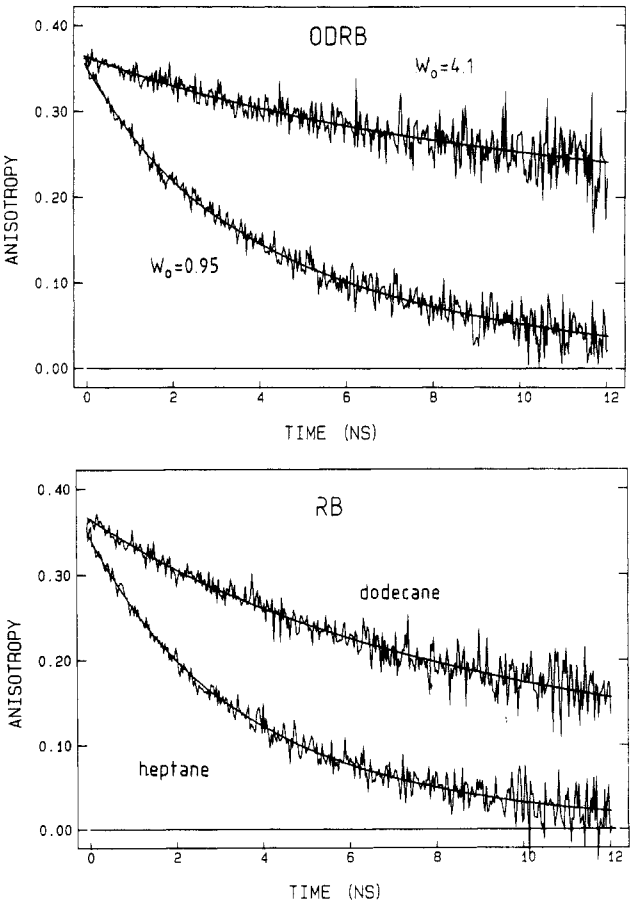

Figure 4. Anisotropy decay profiles and their fits of ODRB (upper panel) and RB (lower panel) in AOT reversed micelles entrapped glycerol. The fits are for biexponential functions with parameters listed in Table III. The upper panel shows that increasing w_0 slows down the micellar tumbling in heptane. The lower panel reveals a similar effect but now for two hydrocarbons with different viscosities.

(33) Fletcher, P. D. I.; Robinson, B. H.; Tabony, J. *J. Chem. Soc., Faraday Trans. 1* **1986**, *82*, 2311.

TABLE IV: Rotational Correlation Times of AOT Reversed Micelles Containing Water and Glycerol in Heptane and Dodecane[a]

w_0	solvent or system	r_h, nm	ϕ_{mic}, ns	ϕ_{obsd},[b] ns	θ_c, deg
			Water		
0.8	H	1.64	1.7	1.9 (2.2)	34 (34)
5.0	H	2.38	5.3	3.4 (2.9)	
16.6	H	4.41	33.6	4.3 (4.0)	
0.8	D	1.64	5.9	6.3 (7.1)	33 (31)
5.0	D	2.38	18.5	7.5 (7.5)	
16.6	D	4.41	117.5	5.7 (6.2)	
			Glycerol		
0.6	RB/H	2.23	4.4	5.4	36
0.95	ODRB/H	2.54	6.4	6.5	30
3.4	RB/H	4.69	40.5	50	20
4.1	ODRB/H	5.31	58.8	59	23
0.6	RB/D	2.23	15.2	23	30
0.7	ODRB/D	2.32	17.2	31	34
1.2	RB/D	2.76	28.9	49	29

[a] For abbreviations see Table I. [b] Values in parentheses are for ODRB.

and only the micellar rotations would appear.

Two examples are shown in Figure 4. In the first example a variation in w_0 is shown from 0.95 to 4.1 for the fluorescence anisotropy decay of ODRB with heptane as organic phase. The fits are for biexponential functions, and the correlation times are listed in Table III. It can be clearly observed that increase of the glycerol droplet lead to distinctly longer correlation times. In this and all other cases an optimum fit is obtained with two correlation times. The shorter correlation time must arise from internal motion similarly as in water droplets, but slower and apparently damped by glycerol. Since the values of the shorter correlation times are, depending on the values of w_0, in the range 2–6 ns, the probe must be located in the interfacial region and not surrounded by viscous glycerol. The longer correlation time must be unambiguously assigned to characteristic times of overall micellar rotation.

The second example in Figure 4 shows the effect of the viscosity of the organic solvent at equal w_0. In this example the value of the long correlation time is dependent on the viscosity of the solvent, as expected for overall, micellar rotation. Also, the internal correlation time increases somewhat, which again is evidence for penetration of organic solvent molecules through the interface formed by the surfactants. In Table III only single fluorescence lifetimes are given. The decay analyses showed that a two-exponential fit yielded slightly better fits with, as for RB in glycerol, a negative, preexponential factor connected to a short lifetime component and a predominant, longer lifetime component. However, the contribution of the short component is far less than observed for RB in glycerol. For the series of experiments as listed in Table III the average value of the negative preexponential factor was -0.05 ± 0.03 and that of the short lifetime was 0.21 ± 0.03 ns. The relatively small preexponential factor is again an indication that the probes are not completely surrounded by the entrapped solvent.

Rotational Dynamics of AOT Reversed Micelles. In Table IV the hydrodynamic radii of both reversed micellar systems are listed as calculated from the empirical relationships (eq 15 and 16) derived previously[25,26] for reversed micelles in heptane. In first approximation we assume that the hydrodynamic radii are the same for dodecane as organic solvent for both water and glycerol droplets. With eq 14 one can calculate the rotational correlation times and compare them with the observed ones. For the reversed micelles with encaptured water there is only agreement between calculated and observed rotation times for $w_0 < 1$ (Table IV), for both heptane and dodecane. At higher w_0 the correlation becomes very poor, which can be ascribed to the predominance of internal rotation.

The agreement between experimental and calculated correlation times is rather good for glycerol droplets in heptane (Table IV). These droplets can be considered as spherical particles, independent of the probe used. With dodecane as hydrocarbon, the observed correlation times of glycerol droplets are consistently longer than calculated by using the radii obtained from reversed micelles in heptane. The latter result can be explained by assuming that the radii of glycerol droplets are effectively larger because of insertion of dodecane molecules between the surfactant molecules. Robinson et al.[34] indeed found a larger water core radius for water droplets with dodecane as hydrocarbon. Also, it cannot be ruled out that the glycerol droplets in dodecane exhibit polydisperse behavior as has been shown by small-angle neutron scattering experiments on water droplets in various hydrocarbon media.[34]

In Table IV we also incorporated the wobbling-in-cone angle θ_c in cases where such a model can be applied, i.e., in small water droplets ($w_0 < 1$) and in glycerol droplets. In the latter reversed micelles the angular displacement is larger at smaller w_0. Such a result can be rationalized by assuming a larger curvature of the surfactants in the smaller micelles, allowing more motional freedom for probes localized in the boundary region.

Concluding Remarks

From time-resolved fluorescence and fluorescence anisotropy measurements on micelles labeled with RB and ODRB, information has been obtained on the polarity of the probe environment and on overall and internal motion of the probe in micelles. The rotational correlation times of the reversed micelles as a whole exhibit good agreement with the calculated rotation times for water droplets at low w_0 and the glycerol droplets in heptane. The glycerol droplets in dodecane are larger than inferred from the hydrodynamic radii derived from the Stokes–Einstein relation. The angular range of internal probe motion is large in small droplets irrespective of probe, entrapped solvent, or hydrocarbon. The amplitude of the restricted probe motion decreases in larger glycerol droplets probably because of a less curved surfactant boundary. Both probes are anchored at the interface between encaptured water or glycerol and the hydrocarbon medium.

Acknowledgment. Part of the work was supported by the Netherlands Foundation of Chemical Research (S.O.N.) with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.). We thank Miss Y. T. Soekhram for preparation of the manuscript.

(34) Robinson, B. H.; Toprakcioglu, C.; Dore, J. C.; Chieux, P. *J. Chem. Soc., Faraday Trans. 1* **1984**, *80*, 13.