never been characterized clearly, but a wealth of indirect proofs favors a β-type structure of the metatungstate ion for this compound.[19] Despite its low stability in aqueous solutions,[20] we succeeded in recording its ^{183}W (Figure 3) spectrum which is comparable to the β-$SiW_{12}O_{40}H_4$ one, i.e., 3 singlets (1:2:1) at δ -107.2 ± 0.1, -120.9 ± 0.1, and -130.6 ± 0.1. This result confirms definitely a β-type structure. Nevertheless, an overnight accumulation gives much broader lines (6-8 Hz, half-width) than with any other heteropolytungstate, this broadening coming either from very temperature-dependent chemical shift or from some exchange process with another intermediate of very low concentration. This broadening process does not allow the determination of the intragroup ^2J coupling, but an extra-group coupling (19 ± 1 Hz) is easily detected on the δ -107.2 (W_I) and -120.9 (W_{II}) lines.

These above examples show how useful can be the determination of $^2J_{W-W}$ couplings. The method will be of particular interest when dealing with multiline ^{183}W NMR spectra one finds, for example, in the 1-11 series. Finally, from Table I[12-15] it is clear that $^2J_{W-W}$ couplings are dependent on the W-O-W angles, but a definite correlation will be forseen only when enough ^2J values are available. We are actually tackling such a project.

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Rotational Diffusion of Rose Bengal in Aqueous Micelles: Evidence for Extensive Exposure of the Hydrocarbon Chains

Wayne Reed, Mario J. Politi, and Janos H. Fendler*

Department of Chemistry, Texas A&M University
College Station, Texas 77843

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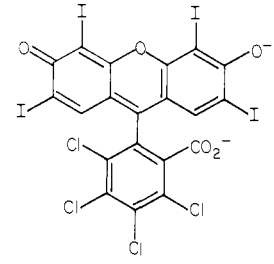
Organization of surfactants in micelles and the extent of water to hydrocarbon contact therein are important and much debated problems. At different times, water was believed to penetrate the micelle completely[1] or not at all[2-4] or reach to any intermediate depth.[5] The classical Hartley model, describing micelles as "oil-droplets in ionic coats",[6] cannot be reconciled with any appreciable water-to-hydrocarbon contact. Three different micellar models have been proposed recently to account for the experimental data. The Menger model describes micelles as porous clusters of surfactants which provide opportunities for considerable water penetration.[7] Fromherz rationalized micellar structures in terms of a surfactant block model which allows wetting the entire hydrocarbon chain in the time average.[8] Dill and Flory discussed molecular organization of surfactants in terms of a statistical theory by using lattice models.[9] This approach provides a substantial probability for methylene groups, even in the middle of the chain, to be in the outer layer of the lattice and predicts a few alkyl chains to lie entirely on the surface of the micelle.[9] Results of kinetic,[10] ^{13}C NMR,[11] and ORD[12] spectroscopic investigations were marshalled in support of the porous micelle model. Interpretation of these results, however, has been questioned.[3] Data are presented in this communication on the rotational diffusion of rose bengal in aqueous micellar hexadecyltrimethylammonium bromide (CTABr) and sodium dodecyl sulfate (SDS) which strongly supports the extensive exposure of surfactant methylene groups to water.



rose bengal

Rose bengal was chosen as a probe since it is a large rigid molecule (a prolate ellipsoid with semiaxes of 2 and 7 Å[13] and a solvated volume of 1670 Å^3 in EtOH[14]) whose absorption and fluorescence spectra, fluorescence lifetime, and anisotropy are extremely sensitive to solvent viscosity and hydrogen bond strength.[14-18]

Rose bengal was purified as described in the literature.[15,19] Its absorption maximum changed from 548 nm in water at neutral pH gradually with increasing amounts of CTABr and SDS to 562 and 558 nm. These data provide evidence for the efficient binding of rose bengal to micelles and allow the assessment of the binding constants, $K_{CTABr} \geq 10^4$ M^{-1}, $K_{SDS} \geq 10^2$ M^{-1}. Fluorescence lifetimes, determined by time-correlated single photon counting using picosecond laser pulses as the excitation source,[20] are given

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(19) Using thin-layer chromatography, emission spectra, and fluorescence lifetimes, we confirmed Cramer's findings.[14,15] Even our purified sample shows, however, a few percent impurity as determined by our extremely sensitive fluorescence lifetime measurements.
(20) A Spectra-Physics cavity dumped rhodamine 6G dye laser, synchronously pumped by a mode-locked argon ion laser (No. 171), was used to provide tunable 15-ps pulses at 400 kHz. Rose bengal samples were excited at 575 nm. The emission, viewed at 90°, was passed through an ultraviolet polacoat polarizer (3M type, 105 UV WRMR) set at 54.7° for lifetime and 0 or 90° for anisotropy measurements. Following the polarizer, the emitted light passed through a Jarrell-Ash 25-cm monochromator, set at 610 nm for rose bengal, onto a RCA 8850 PM tube. The "start" signal for the Ortec 457 TAC was obtained from a portion of the laser pulses via a Texas Instruments TIED 56 silicon avalanche photodiode and an Ortec 437-MHz discriminator. Photon counting and data treatment by the Marquardt algorithm have been previously described.[21] Lack of any instrumental artifact was demonstrated by reproducing the published lifetimes and rotational anisotropies in different solvents (see Table I and ref 25). G values (eq 1) were obtained by measuring the fluorescence intensity of rapidly rotating rose bengal in MeOH at 0 and 90°.
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Table I. Fluorescence Lifetimes (τ) and Rotational Correlation Times (τ_R) of Rose Bengal in Different Media[a]

medium	temp, °C	τ, ps[b]	τ_R, ps
water	25.0	143 ± 40 (118)[c] (95)[d] (78)[e]	<200
methanol	25.0	554 ± 20 (543)[c] (655)[d] (478)[e]	250 ± 50 (190)[f]
1-butanol	25.0		1440 ± 100
propylene glycol	25.0		1930 ± 100
glycerol	25.0		7100 ± 200
1.0×10^{-2} M CTABr	25.0	500 ± 30 (535)[e]	2400 ± 250
1.0×10^{-2} M CTABr	55.0		1110 ± 200
3.0×10^{-2} M CTABr	7.0		3860 ± 350
3.0×10^{-2} M CTABr	10.0		3250 ± 350
3.0×10^{-2} M CTABr	25.0		2540 ± 330
3.0×10^{-2} M CTABr, pH = 4	25.0		2670 ± 300
3.0×10^{-2} M CTABr, pH = 13	25.0		3090 ± 300
3.0×10^{-2} M SDS	10.0	280 ± 15	2540 ± 400
3.0×10^{-2} M SDS	25.0	270 ± 15	1270 ± 370
3.0×10^{-2} M SDS	44.0		910 ± 100
3.0×10^{-2} M SDS	55.0	260 ± 15	<200

[a] [Rose bengal] = $(1-4) \times 10^{-6}$ M. No adjustment made for pH, unless stated otherwise. When pH is given, the hydrogen ion concentration was adjusted by the addition of HBr or NaOH and is determined as bulk pH by a combination electrode. Values in parentheses have been determined in the cited reference. See ref 25 for a discussion of errors. [b] Single exponential, unless stated otherwise. κ^2 ranged between 0.3–3.8 with an average of 2.2. [c] Determined by time-correlated photon counting in ref 15. [d] Determined by a streak camera in ref 16. [e] Determined by a streak camera in ref 18. [f] Determined by time-correlated single photon counting in ref 14.

in Table I.[25] Fluorescence lifetimes of rose bengal in micelles are seen to resemble that in methanol. The probe is unlikely, therefore, to be "buried" in the micelle where it would experience much higher viscosities which would be manifested in considerably longer lifetimes. Indeed rose bengal was suggested to be located at the hydrophilic mantle of CTABr micelles where it is exposed to markedly different protonicity from that of neat water.[18] Solubilization of negatively charged rose bengal in anionic micellar SDS is quite remarkable and shows the overriding importance of hydrophobic interactions.

The emission anisotropy, determined by time-correlated single photon counting,[20] is expressed by

$$r(t) = \frac{I_{\parallel}(t)G - I_{\perp}(t)}{I_{\parallel}(t)G + 2I_{\perp}(t)} \qquad (1)$$

where $I_{\parallel}(t)$ and $I_{\perp}(t)$ are the parallel and perpendicular emission intensities at time t, and G is the calibration factor.[20] It has been shown that $r(t)$ is a single exponential function for spherical and prolate rotators in homogeneous solutions where the transition moment is parallel to the symmetry axis.[16,22] The measured anisotropy in micelles could not be described adequately by a single exponential, particularly for those observed in a longer time window (Figure 1). Rotational correlational times of rose bengal (τ_R), defined as the $1/e$ lifetime of the decay curve, are also given in Table I. Rotational relaxation times of rose bengal in the presence of micellar aggregates may originate in (a) rotation of the unsolubilized probe in water, (b) rotation of the probe in or on the micelle, and (c) rotation of the micelles themselves. The observed extremely short τ_R value in water (Table I) alleviates case a. Temperature dependence of τ_R allowed a distinction between cases b and c. We argued that if the measured anisotropy

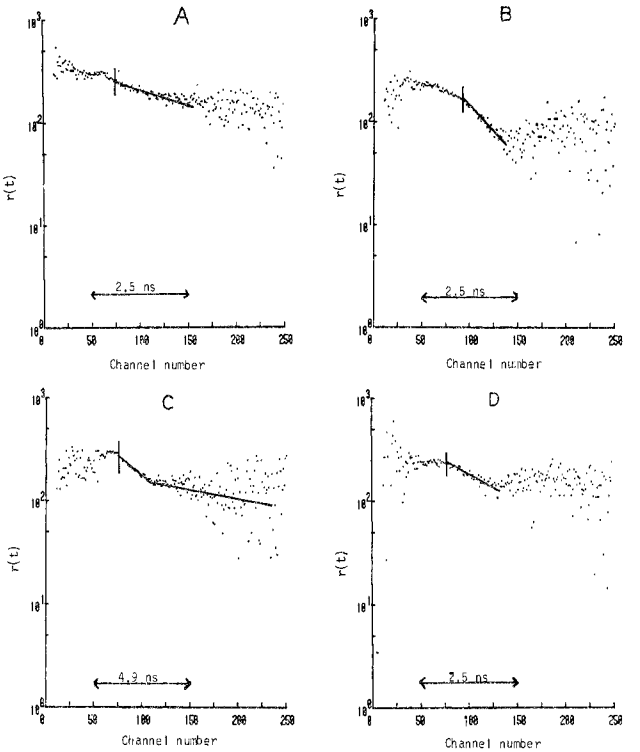

Figure 1. Decay of rose bengal anisotropy in 0.03 M CTABr at 7.0 °C, pH unadjusted (A); in 0.01 M CTABr at 55.0 °C, pH unadjusted (B); in 0.03 CTABr at 25.0 °C, pH 4.0 (C); 0.03 M SDS at 25.0 °C, pH unadjusted (D). Plotted are $r(t)$ values vs. channel numbers (eq 1). Note the differences in real time, indicated on the horizontal bars. Vertical bars are drawn to indicate the beginning of the anisotropy fitting (i.e., after the convolution effect of the laser has passed).

of rose bengal is due to the rotation of micelles in water (i.e., rose bengal merely acts as an indicator for the rapidly tumbling micelle, case c) τ_R should not appreciably change with a change of temperature since the temperature dependence of water viscosity is small and the changes in τ_R caused by inverse temperature dependence would not exceed our error limits over the temperature range investigated. Conversely, microviscosities within or on the surface of micelles are expected to change markedly with temperature. The observed substantial temperature dependence of the main component of τ_R in aqueous CTABr and SDS (Table I) is only compatible with case b. Rose bengal anisotropy at these time scales is entirely due to rotation, parallel to its symmetry axis, in the micellar environment. A similar conclusion has been

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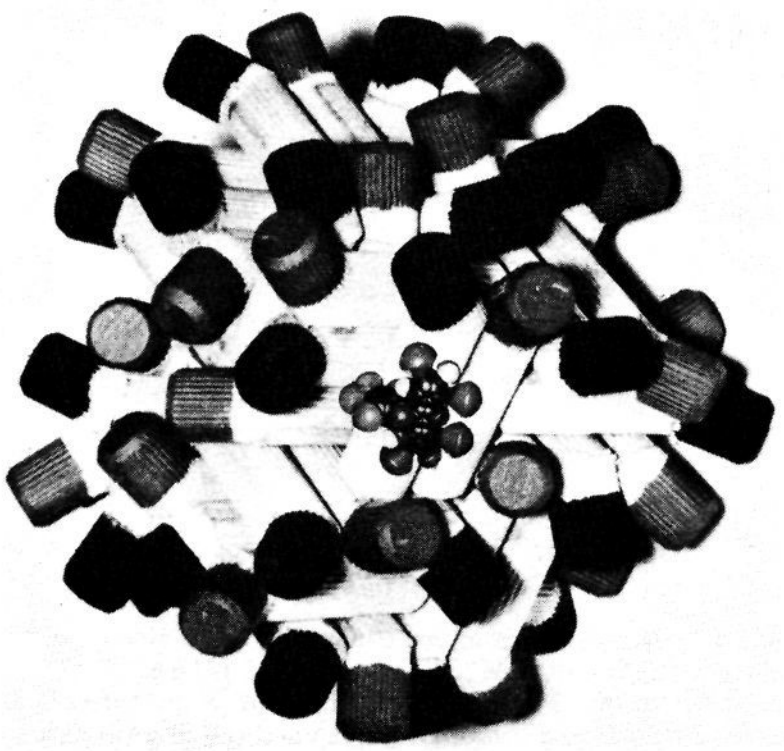

Figure 2. An oversimplified view of the interaction of rose bengal with aqueous micelles. The model is taken from ref 8.[26]

drawn for other rotators in aqueous micelles.[17] If isotropic diffusion and stick boundary conditions are assumed the rotational correlation time is given by the Stokes–Einstein equation

$$\tau_R \equiv 1/(6D) = \eta V/kT \qquad (2)$$

where D is the diffusion coefficient, η is the viscosity of the medium, V is the volume of the rotating molecule, k is the Boltzmann constant, and T is the absolute temperature. Equation 2 allows the calculation of the volume of rose bengal from τ_R and η. Taking τ_R = 2540 ps in 3.0×10^{-2} M CTABr (Table I) and η = 6 cP (extrapolated from a linear plot of τ_R vs. η, determined for rose bengal in various alcohols[14]) R = 7.4 Å and V = 1680 Å^3 are calculated for the radius and volume of rose bengal rotating in the micelle. The excellent agreement of this value with that given in EtOH (V = 1670 Å^3)[14] further supports the proposed mode of rotation (case b) and substantiates the assumed stick-boundary condition.

A second longer lived anisotropy decay of rose bengal was observable in micellar CTABr (see for example Figure 1C). Since this decay is substantially longer than the fluorescence lifetime of rose bengal, τ_R values associated with it can only be approximated. The mean of 8 separate runs for CTABr is 10.6 ± 2.0 ns, and within this experimental error, it is independent of the temperature. This value is most likely to be associated with rotation of the entire micelle (case c). It gives, in conjunction with η = 1.0 (for water), R = 22 ± 5 Å and V = 44 000 Å^3 which approximate the dimensions of the CTABr micelle (R = 35 Å).[23] It should be noted that the emission anisotropy does not approach zero within the time available for its measurements (Figure 1). This, together with the observed second exponential, suggests that rose bengal may only rotate about one of its axes when on the micellar surface, and hence complete depolarization requires the subsequent rotation of the micelle. A similar situation has been encountered for the rotation of diphenylhexatriene in liposomes.[24]

Partitioning of rose bengal in anionic SDS is much less favorable than cationic CTABr. This is manifested in shorter fluorescence lifetimes[25] and greater temperature dependence of τ_R than that observed for CTABr (Table I.). Indeed at 55 °C, τ_R could barely be observed.

The effective incorporation of rose bengal in SDS as well as the lack of appreciable pH effects on τ and τ_R in CTABr (Table I) points to the predominance of hydrophobic interactions. Rose bengal is likely to be "pulled" more strongly in the CTABr than in the SDS micelle as a result of both hydrophobic and electrostatic interactions. This is reflected in longer τ and τ_R, for rose bengal in CTABr, as opposed to SDS (Table I) where hydrophobic forces tend to drag in the probe. Rose bengal is relatively free to rotate in both micelles and experiences an environment similar to alcohol. The Hartley micelle would require the alignment of the rigid rose bengal molecule parallel to the hydrocarbon chain of the surfactant, and concomitant "deep penetration" (consider the radii: 7 Å for rose bengal, 20 Å for SDS, 35 Å for CTABr![23]). Such a solubilization would inevitably lead to the immobilization of the probe and rotational correlation times *entirely* governed by the tumbling of the micelle (case c). Assuming extensive exposure of surfactant hydrocarbons to water is the only way to rationalize the present data. Rose bengal is likely to rotate, relatively unhindered, on the micellar surface where water molecules are hydrating, and in contact with, a fair number of methylene groups. Placing rose bengal on the surfactant block model (Figure 2) provides perhaps the best visualization of the proposed mode of interaction.[26]

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(26) We are grateful to Dr. Fromherz for sending us photocopies of his model.

Fluoromethylglyoxal: Synthesis and Glyoxalase I Catalyzed Product Partitioning via a Presumed Enediol Intermediate

John W. Kozarich,* Ravi V. J. Chari, John C. Wu, and Timothy L. Lawrence

Department of Pharmacology and Developmental Therapeutics Program
Comprehensive Cancer Center
Yale University School of Medicine
New Haven, Connecticut 06510

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Glyoxalase I [*S*-lactoylglutathione methylglyoxal-lyase (isomerizing) EC 4.4.1.5] catalyzes the conversion of the thiohemiacetal **2** of methylglyoxal (**1**) and glutathione (GSH) to the thioester **4** of D-lactic acid and GSH (Scheme I). Studies by Hall, Doweyko, and Jordan[1] have provided evidence for the intermediacy of the enediol **3** initially proposed by Racker[2] and a fast-shielded proton transfer to afford **4** with low incorporation of solvent protons into the C-2 position of the lactoyl moiety. Very recently, Shinkai et al.[3] have demonstrated that 3-methyltetra-*O*-acetylriboflavin inhibits glyoxalase I, presumably by oxidation of the transient enediol intermediate. This is in contrast to the intramolecular hydride shift mechanism proposed by other investigators.[4] We wish to report the synthesis of fluoromethylglyoxal (**5**), a new probe for this enzyme, and our finding that glyoxalase I catalyzes the partitioning of **5** and GSH into *S*-fluorolactoylglutathione and *S*-pyruvylglutathione with concomitant fluoride elimination. These results are consistent with the rapid proton-transfer mechanism and constitute the first use of a fluorinated substrate analogue to evaluate product partitioning via an enediol intermediate.

Fluoromethylglyoxal (**5**) was synthesized according to Scheme II. The dimethylketal **6** of fluorohydroxyacetone was prepared by a modification of the procedure of Pero et al.[5] Moffatt oxidation of **6** (dicyclohexylcarbodiimide/dimethyl sulfoxide/pyridinium trifluoroacetate) in ethyl ether afforded the dimethyl ketal of fluoromethylglyoxal (**7**) in 85% yield.[6] Deketalization

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