Dynamics of 7-Azatryptophan and Tryptophan Derivatives in Micellar Media. The Role of Ionic Charge and Substituent Structure

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We have investigated the motional and population relaxation dynamics of several derivatives of 7-azatryptophan (7AT) and tryptophan (TRP), and *N*-acetyl-tryptophanamide (NATA), in anionic, cationic, and neutral surfactant solutions above the surfactant critical micelle concentrations. We chose the specific derivatives of 7AT and TRP used previously, along with NATA, to elucidate the roles of chromophore charge and side group structure in mediating interactions with micelles. Fluorescence lifetime data are sensitive to local environment, revealing significant differences in the ability of different chromophores to interact with the micelles. Reorientation dynamics of these chromophores indicate persistent probe/micelle interactions that are determined by a balance between ionic charge(s) and dispersion interactions.

Introduction

The dynamics of peptides and proteins in solution is of fundamental importance to many essential biological processes and fluorescence spectroscopy has proven to be a versatile tool for examining such phenomena. The characteristic time scale of fluorescence population decays is matched well to the speed of many important dynamical phenomena in protein and peptide systems.[1] One of the key issues in the study of biological systems is the choice of a fluorescent probe that will not itself serve to perturb the structure of the system under investigation. Tryptophan (TRP) is a widely used chromophore for investigations of peptide and protein behavior because it is one of a limited number of fluorescent amino acids; tyrosine and phenylalanine have been studied less extensively owing to their lower absorption cross sections, fluorescence quantum yields, and the absorption and emission band positions. The indole chromophore present in tryptophan is sensitive to its local environment, and is characterized by an emission maximum between 308 and 355 nm, depending on its immediate environment.

While TRP is found naturally in many biologically active peptides and proteins, it is not an ideal probe because its fluorescence intensity decay is nonexponential, and this anomalous behavior is thought to arise from efficient charge-transfer behavior between multiple stable conformations of the indole chromophore relative to the amino acid moiety.[2] Within the framework of this interpretation, the lifetime of the chromophore is conformation-dependent, making the interpretation of fluorescence lifetime data difficult. In addition, the presence of multiple tryptophan residues in a protein can preclude the extraction of site-specific information from TRP probes.

In recent years, several nonnatural amino acids have been investigated as alternatives to TRP as biological probes, including 7-azatryptophan (7AT).[3−6] The spectral response of 7AT is red-shifted relative to that of TRP, allowing its use in the study of protein structure and dynamics.[7−9] 7AT is characterized by a single-exponential population decay of its S_1 state over a wide pH range, and the time constant of the decay is sensitive to the immediate environment of the chromophore.[3,9] The single-exponential-population relaxation dynamics of 7AT is an expected result for organic chromophores, and its behavior stands in contrast to that of the structurally similar TRP. This difference in relaxation dynamics has been attributed to a relatively low barrier to the formation of a charge-transfer excited-state in 7AT.[10]

We use steady state and time-resolved fluorescence spectroscopy to obtain information on molecules containing the 7-azaindole and indole chromophores in micellar solutions. We are interested determining the effects of chromophore and micelle headgroup charge, and probe molecule structure on their interactions. We use three substituted forms of each chromophore in this comparative study. At neutral pH, they are the zwitterionic amino acids 7-azatryptophan (7AT) and tryptophan (TRP), anionic species formed by the addition of a *tert*-butoxy group at the N-terminus of each amino acid, named boc-7AT and boc-TRP, and cationic species produced though a peptide bond with dodecylamine at the C-terminus of the amino acid residues, designated DD-7AT and DD-TRP. The latter chromophores possess an aliphatic moiety that will allow significant dispersion interactions with micelles. Tryptophan octyl ester has attracted considerable interest as a suitable model for TRP in the case of membrane proteins,[11−15] and the fluorophores DD-7AT and DD-TRP may also serve this purpose. We have also examined *n*-acetyl-tryptophanamide (NATA) in the same micelles. NATA is a neutral species and is of interest because it is an indole-containing molecule that has been used before as a mimic of TRP contained in a peptide backbone and is characterized by a single-exponential S_1 population decay.[16,17] We are interested in micelles because they are a reasonably well-understood class of heterogeneous systems that can function as simplistic models for biomembrane structures. Surfactants and micelles have been investigated extensively due to their many uses, ranging from acting as mediums in which chemical reactions and excitation transport studies can be performed to being used in systems to enhance protein solubility and effecting chemical separations.

We report here on our study of the dynamics of natural and nonnatural amino acid derivatives in aqueous micelles formed by anionic, cationic, and neutral surfactants. Our experimental

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data suggest that probe interactions with the surface or interior of the micelles can dominant, depending on the charge of the chromophore used and the identity of its pendant moiety.

Experimental Section

Materials. The surfactants sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB) and poly(ethylene glycol) 400 dodecyl ether (Thesit) were obtained from Sigma-Aldrich, Inc., checked for fluorescent impurities, and used as received. All chromophores and reagents needed for derivatization were also obtained from Sigma-Aldrich, Inc. The molecules D,L-7-azatryptophan hydrate, D,L-tryptophan hydrate, *N*-acetyl-tryptophanamide, and *N*-*t*-Boc-tryptophan were used as received. *N*-*t*-Boc-7-azatryptophan was prepared by the procedure described by Petrich[6] using D,L-7-azatryptophan hydrate, 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON), and triethylamine in water/dioxane solvent.

DD-7AT and DD-TRP were prepared by condensations of the *N*-*t*-Boc-amino acid with dodecylamine followed by removal of the boc group. The coupling of amino acids to dodecylamine was performed using the water-soluble carbodiimide, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide·hydrochloride (EDCI)[18,19] along with the coupling catalyst hydroxybenzotriazole (HOBt). A description of the coupling and deprotection procedure has been described before. The deprotection procedure was modified slightly. The DD-boc-X compounds (X = 7AT and TRP) were stirred into 9:1 trifluoroacetic acid:dimethyl sulfide, and the progress of the reaction was followed by thin-layer chromatography and worked up as indicated previously.[20]

All aqueous solutions were prepared with water from a Milli-Q water-filtration system. All solutions used were $\leq 5\ \mu$M in chromophore concentration and spectroscopic measurements were performed within 24 h of solution preparation. The sample temperature was controlled using a brass-block cuvette holder maintained at 20.0 ± 0.1 °C (Neslab).

Steady-State Absorption and Emission Spectra. Absorption spectra were acquired using a Cary Model 300 double-beam UV−vis absorption spectrometer set at 2 nm resolution. Emission spectra were recorded with a Spex Fluorolog 3 spectrometer using a 5 nm band-pass for excitation and a 2 nm band-pass for emission collection.

Time-Correlated Single Photon Counting (TCSPC) Spectrometer. The spectrometer we used for the lifetime and dynamical measurements has been described in detail before.[21] The light pulses used to excite the sample are produced by a synchronously pumped, cavity-dumped dye laser (Coherent 702-2, 5 ps pulses and 4 MHz repetition rate). The dye laser is excited by the second harmonic of the output of a continuous wave (cw) mode-locked Nd:YAG laser (Quantronix 416, 100 ps pulses and 80 MHz repetition rate). Samples were excited at their absorption maxima (pyromethene 567 dye, Exciton, KDP Type I SHG). Emission was monitored at the chromophore florescence maximum in the solution of interest using a 20 nm band-pass for collection. Fluorescence was collected at polarizations of 0°, 54.7°, and 90° with respect to the vertically polarized excitation pulse. The instrument response function for this system is typically 35 ps fwhm, and lifetimes measured range from ~600 ps to ~8.5 ns. We did not deconvolute the instrument response function from the experimental data. The shortest reorientation times we measure are on the order of 50 ps, and because we can recover the entire anisotropy decay, loss of the initial portion of the decay does not affect the accuracy of our determinations.

TABLE 1: Solution and Physical Parameters of Micelles Used in This Work

surfactant	charge	viscosity (cP)	pH	conc. (mM)/ no, of cmc	r_h (Å)	τ_M (ns)
CTAB[a]	(+)	1.00 ± 0.01	6.2	2.5/2.7	25.7	17.6
SDS[b]	(−)	1.03 ± 0.01	6.6	25.0/3.0	20.7	9.2
Thesit[c]	neutral	0.99 ± 0.01	7.1	1.0/10	35	44.4

[a] CTAB = cetyltrimethylammonium bromide [b] SDS = sodium dodecyl sulfate [c] Thesit = poly(ethylene glycol) 400 dodecyl ether

Results and Discussion

The objectives of this work are to understand the similarities and differences in the optical response of related chromophores that arise from structural modifications, and to elucidate how these modifications influence probe interactions with their immediate environment. In this study, micelles serve as a heterogeneous solution environment in which to examine these issues. The interactions of these probes with solvent, surfactant, and micelles will be mediated by their charge, substituent structure and functionality, and specific chromophore characteristics. We consider the steady-state and time-resolved spectroscopic data separately, following a brief discussion of solution and chromophore properties important to both groups of measurements.

The surfactant concentrations used were several times their critical micelle concentration (cmc) to ensure micelle formation. To interpret the data and to be certain that changes in the optical responses of the probes are not due to changes in bulk solution properties, several physical parameters and solution properties of the surfactants and micelle solutions must be known. The relevant physical parameters of the surfactant solutions used here are listed in Table 1. The micelle hydrodynamic radius r_h is among the quantities needed to interpret the data. For ionic surfactants, this value is estimated by summing the micelle-core radius, the headgroup radius, and two layers of water.[22,23] This method of determining r_h for ionic surfactants has been found to be in agreement with values obtained from light scattering experiments. The micelles formed by the neutral surfactant Thesit are not as well-ordered as those made using ionic CTAB and SDS amphiphiles. The hydrophilic portion of these two classes of micelles differ significantly. The hydrophilic portion of SDS and CTAB are bulky sulfate and ammonium groups, respectively, while Thesit is comprised of a poly-(ethylene glycol) chain. This latter structural condition results in a hydrated mantle with a less well-defined surface aqueous region, and r_h is estimated at 35 Å.[20]

The dominant forms of the free amino acids TRP and 7AT depend on solution pH due to the presence of carboxylic acid and amine functionalities. Amino acids can take on a positive or negative charge or be zwitterionic, depending on the solution pH. In addition, 7AT can be protonated at the N_7 position, resulting in a significant emission red shift and decreased excited-state lifetime below pH ~5.[24] TRP does not possess a labile proton on its indole moiety, but has been reported to exhibit an additional fluorescence-decay component above pH 9. The existence of multiple different protonated states and low-energy excited electronic states in TRP and 7AT accounts for the sensitivity of their emission spectra to local environment.

Viscosity affects rotational diffusion time constant(s) according to the modified Debye−Stokes−Einstein equation.[25−27] The bulk surfactant solution properties of viscosity and pH were measured at 20 °C with a Canon−Fenske viscometer and a pH meter, respectively. The addition of surfactants at concentrations above their cmc in this study has a negligible effect on solution

TABLE 2: Absorbance, Emission, and Fluorescence Decay Parameters for Probes in Water and Micelles[a]

probe	solution	λ_{abs} (nm)	λ_{em} (nm)	τ_{fl1} (ps)	τ_{fl2} (ps)	α_1/α_2
AT	aqueous	290	399	800 ± 9		
	CTAB	290	399	815 ± 9		
	SDS	290	399	829 ± 9		
	Thesit	290	399	770 ± 9		
boc-7AT	aqueous	290	404	637 ± 9		
	CTAB	290	397	1505 ± 13		
	SDS	289	404	642 ± 11		
	Thesit	289	404	660 ± 12		
DD-7AT	aqueous	290	397	901 ± 10		
	CTAB	290	397	1467 ± 11		
	SDS	290	384	578 ± 9		
	Thesit	290	387	924 ± 11	2407 ± 70	9.4
TRP	aqueous	279	359	872 ± 100	3470 ± 50	0.11
	CTAB	279	359	634 ± 47	3384 ± 80	0.13
	SDS	279	359	885 ± 150	3100 ± 70	0.15
	Thesit	279	359	689 ± 68	3473 ± 80	0.11
boc-TRP	aqueous	279	363	1033 ± 39	8450 ± 26	0.14
	CTAB	282	352	1858 ± 33	5923 ± 33	0.45
	SDS	279	363	792±36	8361±18	0.25
	Thesit	279	363	1000±27	8383±20	0.27
DD-TRP	aqueous	279	351	1849±109	3049±102	0.49
	CTAB	281	351	1253±32	3634±56	0.59
	SDS	279	339	948±35	2827±25	0.52
	Thesit	281	345	2849±113	6783±192	1.67
NATA	aqueous	279	360	3153±18		
	CTAB	279	358	3067±22		
	SDS	279	358	1035±90	2746±40	0.2
	Thesit	279	360	3168±60		

[a] The α-ratio (last column) is the ratio of the prefactors for the two decay components

bulk viscosity. We measure changes in pH in the solutions, but these pH changes do not occur in a region that affects the state of protonation of the chromophore or the overall charge of probe molecules significantly. The changes in bulk solution properties associated with the formation of the micelles are small and will not influence the optical response of the chromophores substantially.

Steady-State Spectroscopy. The experimental absorbance and emission maxima of the chromophores studied here are given in Table 2. Representative emission spectra are shown in Figure 2 for the TRP derivatives in SDS and Figure 3 for boc-AT in water and aqueous micelle solutions. Inspection of the absorbance maxima indicate the ground states of the chromophores are not perturbed to a great extent by covalent modification of the chromophore side groups or by the presence of micelles in solution. The structure- and environment-dependent changes in emission maxima for these chromophores are more pronounced. Both 7AT and TRP are known to exhibit emission spectral shifts when incorporated into peptides and proteins, with the spectral shifts seen for TRP being larger than those for 7AT. The first comparison we make is between the free residues, the *N*-boc-modified chromophores, and C-terminus-modified dodecylamine forms, all in water.

Compared to the free residues, emission of the boc-modified forms are red shifted by 5 nm (7AT) and 4 nm (TRP), and the dodecylamine-modified chromophores are blue-shifted by 2 nm (7AT) and 8 nm (TRP). The spectral shifts we observe for these chromophores arise from substituent effects on the excited states of the chromophores. It is known that the charged groups of zwitterionic TRP influence its excited state(s) and that the emission shifts we observe are related to the stabilization of different charge distributions on the substituted molecules.

In micellar solutions, the behavior of 7AT and TRP are similar to that seen in aqueous solutions. The steady-state

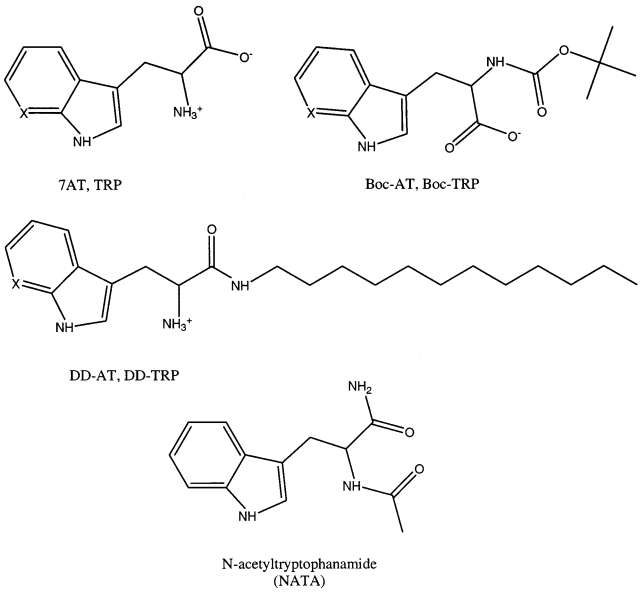

Figure 1. Structures of the chromophores in this study. For 7AT X=N and X=C for TRP.

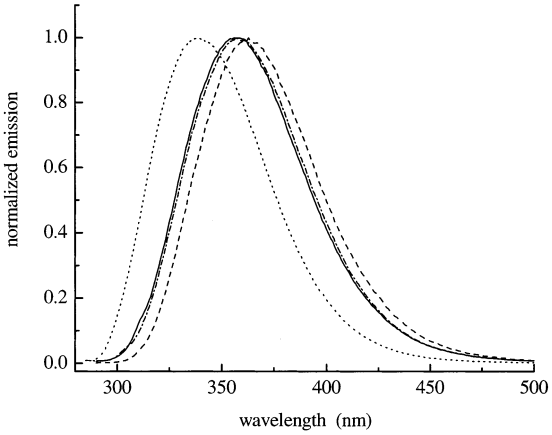

Figure 2. Normalized emission spectra of tryptophan derivatives in 25 mM SDS solution. TRP = solid line, boc-TRP = dashed line, DD-TRP = dotted line, NATA = alternating dot/dash line.

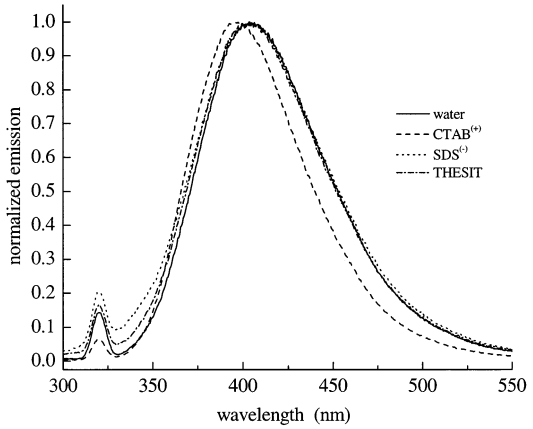

Figure 3. Normalized emission spectra of boc-7AT in aqueous micelle solutions and water. boc-7AT in water = solid line, CTAB solution = dashed line, SDS solution = dotted line, Thesit solution = alternating dot and dashed line.

responses of the amino acids show no change in emission maxima in micellar solution, indicating that the presence of the micelles is not altering their local environment to a significant extent. The boc-modified derivatives are anionic above ~pH 3

by virtue of the presence of a free carboxylate functionality, and exhibit emission blue shifts relative to water in solutions containing the cationic micelle CTAB. DD-7AT and DD-TRP are cationic below pH 9 and these chromophores also exhibit significant blue shifts in solutions containing anionic SDS micelles. This is an expected result, with blue shifts being seen for indole-based ionic chromophores interacting with micelles containing oppositely charged headgroups.[28,29] The steady-state emission data of the chromophores we report on here are thus useful for gauging interactions between the chromophores and micellar structures in solution.

Fluorescence Lifetimes of 7AT, TRP, and NATA. Excited-state lifetime measurements can provide information on solution composition and may also contain information on the interactions between the probe and the micelle. For the same reason that the emission spectra of the chromophores are sensitive to local environment, their fluorescence lifetimes reflect intermolecular interactions. 7AT is zwitterionic at pH 7, and its emission intensity decays as a single exponential with a time constant of ~800 ps. As noted above, 7AT has been incorporated into peptide fragments and oligomers, and its excited-state population decays as a single exponential in uniform environments, with the exception of the octapeptide, as reported by Petrich.[30] The excited-state population decay of TRP is more complex. TRP typically displays a biexponential fluorescence intensity decay in its zwitterionic form and when incorporated into peptides and proteins. NATA has been used as a simple model for tryptophan incorporated into a peptide backbone. This is not necessarily a good model since NATA is anomalous among indole-containing molecules; it is known to exhibit a single-exponential fluorescence intensity decay. We consider the population relaxation dynamics of these three chromophores separately.

7AT Derivatives. The fitted parameters for the emission-intensity decays of the probes used in this study are listed in Table 2. All fluorescence lifetimes fit well to either a single- or a double-exponential decay. Goodness-of-fit was evaluated by χ^2 criterion and visual inspection of the residuals of the fitted function to the data. A single-exponential intensity decay indicates that the fluorescent species exists in a single environment or that there is rapid exchange between multiple environments over the time scale of the measurement. Deviations from single-exponential decay behavior indicate the fluorescent species exists in multiple stable environments where exchange is slow, or simultaneous relaxation from multiple excited electronic states. Probe molecules containing the 7-azaindole chromophore exhibit a time-domain response fitted best to a single-exponential decay, save for DD-7AT in Thesit. Inspection of the lifetime data reveals that the lifetime of zwitterionic 7AT depends little on the presence of or proximity to micelles, suggesting that 7AT experiences a similar environment in water and micellar solutions and that it does not interact appreciably with surfactant molecules or micelles. Modifying the amino and carboxylic acid functionalities of 7AT affects the steady-state emission properties of the chromophore and has a pronounced effect on the fluorescence lifetime. In water, the fluorescence lifetimes range from ~640 ps for boc-7AT to ~800 ps for 7AT and ~900 ps for DD-7AT.

The fluorescence decays for boc-7AT in water, SDS, and Thesit are similar, but in CTAB, the lifetime more than doubles to 1.5 ns. It is unusual to recover lifetimes this long for the 7AT chromophore in aqueous media; the 1.5 ns lifetime is consistent with the lifetimes seen for 7-azaindole in nonpolar systems.[31,32] Boc-7AT exhibits an emission spectral blue shift in CTAB relative to water, consistent with the chromophore residing in a nonpolar environment. For boc-7AT in CTAB there is no evidence of dual emission behavior, seen in neat aliphatic solvents,[31] and it would not be expected unless the chromophore were sequestered in the center of the micelle, since water is known to penetrate significantly into the mantle of ionic micelles.

The lifetime of DD-7AT depends sensitively on its local environment. In SDS micelles, DD-7AT has a lifetime of ~580 ps. Its lifetime increases to ~1.5 ns in CTAB, nearly the same as for boc-7AT in CTAB, with which DD-7AT shares an essentially identical steady-state emission response. This finding suggests that the substituted chromophores may reside in similar environments and states of solvation despite their opposite charge and different pendant structures. DD-7AT displays a two component fluorescence decay in the neutral micelle Thesit, with the time constants of 924 ps (90%) and 2.4 ns (10%). There are several possible explanations for these data. The first is that we are measuring partitioning between free and micelle-bound probes, based on the fast decay component being similar to that of DD-7AT in water. For DD-7AT in Thesit, the steady-state emission is shifted 10 nm with respect to water. The second possibility is that there are several environments in a Thesit micelle, and these data could be accounted for in the context of exchange between the micellar environments.

TRP Derivatives. The fluorescence-intensity decay of TRP is known to be biexponential, with time constants of ~0.8 and ~3.5 ns in water. When TRP is placed in micellar solutions, only small changes in the time constants are observed. This finding suggests that the presence of micelles has a limited effect on the chromophore excited state, similar to our findings for 7AT. The fluorescence-intensity decay of boc-TRP in water is also biexponential, with time constants of ~1 ns and ~8.5 ns (Table 2). The slow-decay time constant is significantly longer for boc-TRP than for TRP and one explanation for this finding is that the TRP long lifetime is shortened due to self-quenching by the charged amino group at neutral pH. The lifetime of boc-TRP is similar in water, SDS, and Thesit but with time constants of ~2 ns and ~ 6 ns in CTAB. For boc-TRP, the short-lifetime component shows a significant increase in lifetime in CTAB, like the 7AT derivatives, while the long component decreases with respect to its value in water. While the interactions of boc-TRP are likely mediated by electrostatic forces, interpretation of its lifetimes is complicated by the complex and opposing manner in which the two lifetime components change with environment.

DD-TRP displays the most environmentally sensitive fluorescence response, with unique fluorescence-decay time constants for each system measured. The decay times of boc-TRP and DD-TRP do not appear to be correlated to one another, in contrast to the data for boc-7AT and DD-7AT. In comparison to the other indole containing species, the fluorescence response of DD-TRP in Thesit is the most unusual as was DD-7AT for the azaindole probes. The short- and long-time components of DD-TRP in Thesit are about twice their value(s) in water (Table 2). In CTAB, the short time constant decay comprises the majority of the fluorescence decay, in contrast to the behavior seen for 7-azaindole-based probes. We note that this anomalous behavior is not seen for the reorientation data (vide infra) and further investigation will be required to identify the basis for these findings.

NATA. The fluorescence of the neutral chromophore NATA exhibits single-exponential decay kinetics with a decay time constant of ~3.1 ns in water, CTAB, and Thesit (Table 2). In

SDS-containing solutions, NATA exhibits a two-component population decay. The lifetimes recovered for NATA and DD-TRP in SDS are similar. We believe this situation to be coincidental; the indole chromophores are not experiencing the same environment, as seen by the large difference in the steady-state emission (19 nm) and the relative contributions of each lifetime component for these two probes.

Rotational Diffusion Measurements. To better understand the interactions between the probes and micelles, we have investigated the rotational diffusion dynamics of the chromophores in aqueous solutions of micelle-forming surfactants. Time-resolved fluorescence−depolarization experiments provide information on the rotational diffusion of the probe molecules. These measurements are sensitive to and can provide information about intermolecular interactions over the length scale of the probe molecule.[33,34] Reorientation data provides direct information on probe-surfactant association. We can distinguish whether probes are interacting significantly with micelles by comparing the motional dynamics of the probes in water and micellar solutions.

There are many studies in the literature on the rotational diffusion of organic chromophores in neat and selected binary solvent systems.[34,35] Micellar environments have been examined before[36−40] and are a special subset of binary systems. The theory of rotational diffusion is well-established, and because the relevant molecular processes proceed on a time scale that can be accessed experimentally, it has found wide use as a means of probing intermolecular interactions and transient solution-phase organization. The characteristic time scale of the events determining reorientation dynamics are short, and as a result, many interactions occur over the course of the chromophore's reorientation, resulting in the overall experimental observation being the average molecular environment experienced by the probe. For reorientation measurements in binary solutions it is often found that the molecular-scale interactions that mediate the probe motion are not reflective of the bulk properties of the solvent. In polar systems, the relevant intermolecular interactions may include dipolar interactions, dielectric friction,[41] and under certain circumstances, and formation of solvent/solute complexes. Our measurements point to the existence of strong interactions between certain of the probes and the micelles and we interpret our reorientation data in the context of a hindered rotor model.

We use a pulse of vertically polarized light to excite an ensemble of chromophores in solution and monitor the time-dependent decays of the polarized components of emission. We combine the emission-intensity data polarized parallel and perpendicular to the excitation polarization and generate the induced orientational anisotropy function $R(t)$

$$R(t) = \frac{I_{||}(t) - I_{\perp}(t)}{I_{||}(t) + 2I_{\perp}(t)} \qquad (1)$$

where $I_{||}(t)$ and $I_{\perp}(t)$ are the time-dependent emission intensities. The function $R(t)$ represents the rerandomization of the ensemble of excited molecules. In these measurements the data are compared to various models in an effort to relate the functionality of the $R(t)$ decay to the immediate environment of the chromophore. Theoretically, $R(t)$ can contain up to five exponential decays and its form depends on the shape, size, flexibility of the labeled molecule, and the angle between the transition moments of the chromophore.[42] In simple homogeneous environments, a single-exponential decay is commonly recovered and it is unusual to observe more than two decays. The anisotropy of all the chromophores we examine in this study decay by a single exponential in water and the anisotropy decay of DD-AT in water is shown in Figure 4. These same probes displayed marked changes in their anisotropy decay in at least one type of micelle solution with exception of the free amino acids, which remain single exponential. The experimental anisotropy functions are seen to change from single decays to decays with two exponential components (see Figure 5) in micellar solutions,

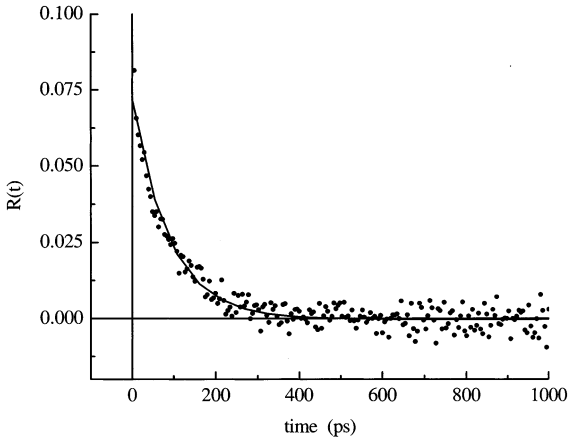

Figure 4. Experimental anisotropy decay of DD-AT in water (data points) and best-fit line to a single-exponential decay function.

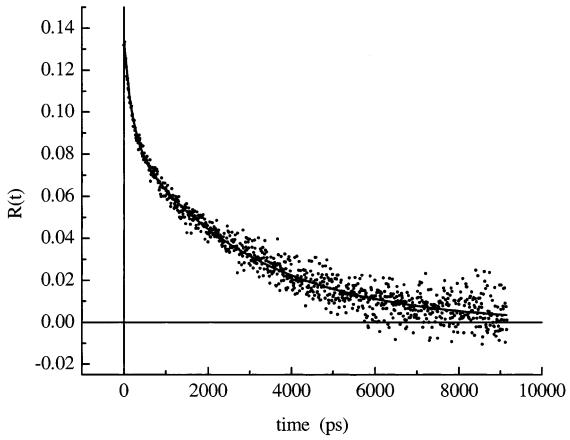

Figure 5. Experimental anisotropy decay of DD-AT in Thesit solution (data points) and best-fit line to a biexponential decay function.

$$R(t) = R_1(0)\exp(-t/\tau_1) + R_2(0)\exp(-t/\tau_2) \qquad (2)$$

and a central issue is assigning the origin of the biexponential behavior. It is significant that we observe different functionalities for the anisotropy decay in water and in micellar solutions. This finding points to the interaction of the probe(s) with the micelles and we consider two different models to account for our experimental findings.

In water, a one-component reorientation decay is consistent with our earlier findings for AT derivatives[20,24] as well as one-component decays reported for tryptophan and NATA.[43] These simple decay data may be interpreted in the context of the modified Debye−Stokes−Einstein (DSE) model.[25−27]

$$R(t) = R(0)\exp(-t/\tau_{OR})$$

$$\tau_{OR} = \frac{\eta V f}{k_B T S} \qquad (3)$$

where τ_{OR} is the reorientation time constant, η is the solution bulk viscosity, V is the solute hydrodynamic volume,[44] f is a friction coefficient to account for the solvent/solute boundary condition, and S accounts for the nonspherical shape of the volume swept out by the reorienting molecule.

We now consider the reorientation of the probes in micellar solutions. Comparing the reorientation data gathered in water to the data for the micellar solutions allows us to determine whether the chromophores are associating or incorporating in some manner into micelles. In cases where a single-exponential anisotropy decay is seen in micellar solution, the location of the chromophore can be open to question. We assert a change in the functionality of the anisotropy decay in micellar solutions indicates incorporation into micelles. We observed that certain probes display biexponential anisotropy decays, signaling incorporation into micelles, and we need to choose an appropriate model to aid interpretation of the observed dynamics.

The fluorescence depolarization of a probe molecule associated with a micelle results from both the rotation of the micelle and the molecular dynamics of the probe associated with the micelle. The rotation of the micelle in solution is independent of the dynamics of the associated probe molecule. The probe dynamics may include movement of the probe in a restricted region of the micelle and translational motion of the adsorbed or tethered probe in or across the surface of the micelle. These dynamics depend sensitively on probe and micelle molecular structure.

There are several models that may be applicable to the interpretation of $R(t)$ decays in an environment where rotational motion is hindered.[45−48] $R(t)$ can be predicted to decay as a single exponential in the following two cases. The anisotropy decay time constant represents the motion of the (spherical) micelle in the case where the chromophore is attached to the micelle as a rigid entity and this motion is characterized by a time constant τ_M. In the second case, the chromophore is located in the core of a micelle and is free to rotate within that confined volume. In this model, the anisotropy decays with a time constant of $(\tau_M^{-1} + \tau_R^{-1})^{-1}$, a single exponential, where τ_R is the decay time constant associated with chromophore motion in the core of the micelle. The experimental time constants we recover and their magnitudes suggest that neither model is appropriate for the systems we report on here.

The time constants and zero-time anisotropies for reorientation of the probes in water and the micelles SDS, CTAB, and Thesit are given in Table 3. The zwitterionic species 7AT and TRP exhibit single exponential anisotropy decays in all solutions. There are measurable differences in the reorientation time constants for these probes in surfactant solutions, and these differences cannot be accounted for simply by changes in bulk solution properties; the bulk viscosities of the micelle solutions are essentially the same as that of water. There must be some interaction of 7AT and TRP with the micelles, but the nature of the interaction and its persistence time cannot be addressed directly by these data alone. The single-exponential anisotropy decays seen for these chromophores suggest that the persistence time of any probe/micelle interaction is short relative to the reorientation time of the micelle.

The reorientation dynamics of substituted 7AT and TRP, and with NATA in several of the micelles are fundamentally different than those seen for TRP and 7AT. For the oppositely charged probe/micelle systems (boc-X^-/$CTAB^+$ or X-DD^+/SDS^-), the experimental $R(t)$ function is a biexponential decay. It is tempting to interpret this functionality as representing the interactions between probe and micelle being facilitated by ionic interactions. While such interactions are likely important, we see evidence in the data that (neutral) NATA also interacts significantly with ionic micelles. Thus ionic interactions alone cannot account for the probe/micelle interactions. Additionally, when the chromophores have structural features in common with the surfactants, such as C_{12} aliphatic chain, ionic interactions appear not to dominate the probe/micelle interactions. This finding is consistent with the existence of a double-layer structure at the interface between amphiphile headgroups and the bulk medium. Screening from such a double-layer structure can serve to mediate the importance of ionic interactions.

TABLE 3: Fitted Decay Time Constants and Zero-Time Anisotropies for the Systems Indicated in the Left Column. The Experimental Data Fitted to the Function $R(t) = R_1(0) \exp(-t/\tau_1) + R_2(0) \exp(-t/\tau_2)$[a]

probe	solution	$R_1(0)$	τ_1 (ps)	$R_2(0)$	τ_2 (ps)
AT	aqueous	0.063 ± 0.01	41 ± 5		
	CTAB	0.065 ± 0.005	63 ± 8		
	SDS	0.07 ± 0.005	54 ± 5		
	Thesit	0.064 ± 0.005	50 ± 4		
boc-7AT	aqueous	0.061 ± 0.005	63 ± 8		
	CTAB	0.041 ± 0.003	256 ± 18	0.05 ± 0.002	2122 ± 120
	SDS	0.064 ± 0.003	66 ± 6		
	Thesit	0.06 ± 0.005	73 ± 7		
DD-7AT	aqueous	0.066 ± 0.002	98 ± 5		
	CTAB	0.033 ± 0.002	196 ± 17	0.031 ± 0.002	1337 ± 97
	SDS	0.032 ± 0.004	85 ± 19	0.046 ± 0.004	498 ± 47
	Thesit	0.047 ± 0.002	196 ± 10	0.088 ± 0.002	2975 ± 81
TRP	aqueous	0.09 ± 0.01	51 ± 5		
	CTAB	0.1 ± 0.01	70 ± 7		
	SDS	0.08 ± 0.01	67 ± 7		
	Thesit	0.08 ± 0.01	47± 6		
boc-TRP	aqueous	0.11 ± 0.02	80 ± 8		
	CTAB	0.05 ± 0.01	146 ± 21	0.07 ± 0.01	1341 ± 166
	SDS	0.14 ± 0.02	124 ± 10		
	Thesit	0.082 ± 0.02	101 ± 15	0.068 ± 0.02	529 ± 0.04
DD-TRP	aqueous	0.09 ± 0.01	118 ± 15		
	CTAB	0.051 ± 0.005	205 ± 17	0.052 ± 0.005	1213 ± 56
	SDS	0.06 ± 0.008	223 ± 25	0.05 ± 0.005	1557 ± 66
	Thesit	0.035 ± 0.004	207 ± 43	0.09 ± 0.003	1204 ± 57
NATA	aqueous	0.1 ± 0.01	72 ± 7		
	CTAB	0.11 ± 0.01	71 ± 7		
	SDS	0.09 ± 0.01	76 ± 12	0.04 ± 0.006	630 ± 86
	Thesit	0.09 ± 0.01	73 ± 10		

[a] For probe/solution systems with one decay constant indicated, $R_2(0) = 0$

The anisotropy functions of the cationic probes DD-7AT and DD-TRP exhibit two-component exponential decays in all micellar solutions. The existence of two reorientation time constants could result either from the probe partitioning into two distinct environments or from the probe residing in a single environment and the two-component reorientation dynamics resulting from the effective rotor shape of the probe. An examination of the fluorescence lifetime data in conjunction with the reorientation data allows these possibilities to be distinguished. The azaindole-containing probes exhibit single-exponential emission-intensity decays in water. The fluorescence intensity decay of DD-7AT in Thesit displays a major component (90%) with a time constant of 924 ± 11 ps and a minor component (10%) with a time constant of 2407 ± 70 ps. The short component for this system is the same as that recovered in pure water, 901 ± 10 ps. The lifetime data suggest a small fraction of probe being incorporated into micelles, with the majority of the probe in water. Thus the reorientation data are indicative of the probe partitioning into two environments; one on or within the micelle and the other dominated by bulk water. The fact that the fast reorientation time of DD-7AT in the micellar system (∼200 ps) is twice that measured in water (∼100 ps) indicates that the probe resides in an aqueous environment that is influenced substantially by the presence of

the micelles. It is possible that the fast reorientation time constant for this system is the result of rapid association/dissociation kinetics for probe/micelle interactions. DD-7AT exhibits a two component anisotropy decay in CTAB and SDS micellar solutions, as does boc-7AT in CTAB. The variations in anisotropy decay time constants for these systems point toward there being significant and relatively short-lived probe interactions with the micelles.

The analogous TRP derivatives display double-exponential anisotropy decays in micellar solutions. The recovered time constants for the indole and azaindole probes show similar trends but considerably different time constants in their reorientation dynamics. Intuition suggests that these chromophores would exhibit similar reorientation behavior. The structural similarity of TRP and 7AT derivatives is central to the use of 7AT as an alternative to TRP for probing biological systems. While 7AT has been used effectively in situations where biological activity is retained, the rates of activity changed significantly upon substitution. Another indication of the significant differences 7AT-substitution introduces into biological environments is the experimental finding that certain 7AT-substituted proteins can be crystallized, where the corresponding nonmutant tryptophan-containing proteins have not been crystallized.[49] It appears that the presence of the ring-bound N at the 7-position in 7AT plays an important structural as well as spectroscopic role in determining the properties of this molecule.

When two decays are seen in a micellar system, it is reasonable to assume that the decays correspond to fast, restricted motion within the micelle or at its surface, and slower global motion of the micelle. The recovered short-time constants span a range from 85 to 260 ps and the long components vary from 0.5 to 3 ns. The probes are not bound rigidly in the micelle or free to rotate within the core because they do not display single-exponential dynamics or possess time constants consistent with these scenarios. The short component depends on the specific probe/micelle system and the time constants recovered are not inconsistent with those expected for restricted motion in or on a micelle. For all of the systems we report here, the long-decay-time constant never approaches that expected for global micelle motion if the probes are attached rigidly to or contained within a micelle. The correlation times expected for the micelles τ_M are listed in Table 1. These correlation times were calculated using eq 3 in the stick-limit ($f = 1$; $S = 1$), where r_h is the radius of the micelles, used to determine volume. These predicted correlation times range from several nanoseconds to tens of nanoseconds, depending on the size of the micelle. The slow time constants we measure experimentally are not consistent with the motion of the free probes. These findings leave quasitranslational motion of the chromophore along the outer extent of the micelle as the only physical motion consistent with this time constant.[20]

Owing to the structures of the probes and micelles used here, there are two significant types of interactions that can occur. These are ionic and dispersion interactions, and they can act either in concert with one another or in an opposing manner, depending on the specific system. It is evident from the experimental data that there is substantial interaction between the oppositely charged probes and micelles and when the probes share common structure with micellar constituents. For the cationic probes DD-7AT and DD-TRP, we observe qualitatively similar behavior in cationic, anionic and neutral micelles, with the differences in the time constants between systems likely being related to the characteristic persistence times for the dominant interactions. We take these findings to indicate that for C_{12} aliphatic chains interacting with micelles of similar length, the dispersion forces compete effectively with ionic forces and dominate the observed dynamics. This finding can be reconciled if we consider that the nominally charged probe and micelle moieties are likely screened efficiently by spectator ions, leaving dispersion interactions as the dominant forces in these systems. For the anionic boc-7AT and boc-TRP derivatives, we recover two-component anisotropy decays in cationic CTAB micelles, single-component decays in anionic SDS micelles and in water, and probe-dependent behavior in the neutral Thesit micelle. We understand the behavior of these probes in CTAB and SDS as being mediated primarily by ionic interactions, and the different behavior of the two probes in Thesit results from the subtle structural difference between the chromophores. We have discussed this issue above. The neutral probe NATA appears to remain predominantly in the aqueous phase of all micellar systems, with measurable contributions from micelle-incorporated chromophore being seen only for SDS.

We apply a single formalism to the systems that exhibit two-component anisotropy decays to explain their physical behavior. The fundamental physics responsible for the data we observe is essentially the same for all of the micellar systems, with differences in the details being associated with the specific identities of the micellar constituents. We summarize our findings as follows: (1) 7AT and TRP exhibit only small changes in τ_{OR} for each surfactant solution compared to their behavior in water, indicating that the probe molecule itself does not associate substantially with the micellar structures. This finding is corroborated by the lifetime and steady state emission data. (2) The number of anisotropy decays seen for any of the probes studied here is an indicator of the extent of probe/micelle interactions for that system. Two-component anisotropy decays indicate probe/micelle interactions and one-component anisotropy decays suggest that the probe resides primarily in the aqueous environment. (3) The details of the probe/micelle interactions are determined by the balance between ionic and dispersion interactions, where the double layer serves to attenuate ionic interactions.

We consider next the time constants we obtain experimentally and how to reconcile these data with the structural features of the systems we have investigated. For the case where the chromophore is bound near the surface of the micelle, a biexponential decay is expected, with time constants τ_w arising from restricted motion analogous to that of a hindered rotor, and $_M$ for overall reorientation of the micelle.[47] If translational motion of the chromophore about the surface of the micelle is considered, a third decay component τ_d is expected.[36] The anisotropy function derived from this model is given by eq 4,

$$R(t) = R(0)[S'^2 + (1 - S'^2)\exp(-t/\tau_W)] \exp(-t(1/\tau_d + 1/\tau_M)) \quad (4)$$

where $R(0)$ is the zero-time anisotropy and S' is an order parameter which is a measure of the equilibrium orientational distribution of the chromophore transition moment. Because the micelles are relatively large the additional orientational relaxation imposed on the probe only affects the slow decay component due to translational diffusion. These motions take place independently and their decay rates can be added; thus, eq 4 does not include these motions explicitly. Depending on the complexity of the model used in the interpretation of the experimental data, it is possible to over-interpret the information present. We are cognizant of this possibility and we choose to use the simplest model that accounts for the features of the data.

The expected reorientation time constant for a micelle is several nanoseconds and we do not observe any such long-time anisotropy decay in our data. The possible reasons for this finding are either that our measurements are not sensitive to such a long-time component, which is not the case, or that the persistence time of the micelle/probe association is substantially shorter than the reorientation time of the micelle. The latter explanation is consistent with interactions between the probe and the micelle that are characterized by a relatively small driving force. We consider that the interaction between the probes and the micelles is dominated by ionic interactions for the smaller charged probes and by dispersion-mediated penetration of aliphatic moieties into the lipophilic portion of the micelle for DD-7AT and DD-TRP. In all cases, the chromophore is in closest proximity to the micelle headgroups, and this portion of the probe is capable of motion at or near the surface of the micelle. We treat this motion as translational diffusion on a spherical surface. Equation 4 describes the general case of the anisotropy decay for three independent motions and we can apply the following simplifications to bring this model into consistency with the experimental data,

$$1/\tau_d = 1/\tau_{slow} - 1/\tau_M \quad (5)$$

$$1/\tau_w = 1/\tau_{fast} - 1/\tau_d - 1/\tau_M = 1/\tau_{fast} - 1/\tau_{slow} \quad (6)$$

We determine values of τ_M from the DSE equation at 20 °C, or we assume $\tau_M = \infty$, but in either case we are insensitive to this quantity experimentally. The translational diffusion coefficient D_t can be related to τ_d through the radius of the micelle r_h (Table 1) over which the probe diffuses, by eq 7.[38]

$$D_t = r_h^2/6\tau_d \quad (7)$$

This diffusion constant is related to the slower of the two motions we sense. The fast time constant is related to the rotational motion of the chromophore about its tethering bond to the micelle. The wobbling-in-a-cone model[45,47] assumes the molecule is constrained within a cone of semiangle θ_0 with the rotational motion of the molecule being described by a wobbling diffusion coefficient D_w. The value of D_w is related to our experimental data through the quantities τ_w and S',

$$D_W = \{\tau_w(1 - S'^2)\}^{-1}[-\cos^2\theta_0(1 + \cos\theta_0)^2\{\ln[(1 + \cos\theta_0)/2] + (1 - \cos\theta_0)/2\}\{2(1 - \cos\theta_0)\}^{-1} + (1 - \cos\theta_0)(6 + 8\cos\theta_0 - \cos^2\theta_0 - 12\cos^3\theta_0 - 7\cos^4\theta_0)/24] \quad (8)$$

where the cone semiangle θ_0 is related to the order parameter through

$$S' = 0.5\cos\theta_0(1 + \cos\theta_0). \quad (9)$$

The values for S', τ_{slow}, and τ_{fast} were extracted from the experimental data using eq 4, and from this information, the quantities θ_0, D_w, and D_t were calculated for each surfactant system (Table 4). The values of D_w for probes yielding single-exponential decay were calculated using $D_w = (6\tau_{OR})^{-1}$. The order parameters in these solutions are zero and the equilibrium orientational distribution is random because the probes are assumed to reorient freely.

The values of the order parameter S, vary from 0.56 to 0.85 for reorientation of the probes in the micelles. The order parameter is a measure of the time-averaged orientational distribution of the chromophores and can take on values from zero for a fully random orientational distribution, to one for rigid, highly ordered systems such as crystals. The values recovered for the order parameter indicate the probes do have preferential orientation with respect to the micelle surface. On the basis of the S' values we extract from our data, the most motionally restrictive environment is formed by Thesit for the probes DD-TRP and DD-7AT. These probe/micelle systems displayed among the largest changes in reorientation times compared to their behavior in water. These probes likely interact significantly with the poly(ethylene glycol) mantle of the Thesit micelles. The lowest value of S′ is seen with the NATA/SDS system. NATA/SDS also yields the largest wobbling diffusion coefficient, approaching that of NATA in water. These complementary pieces of information point to NATA being only weakly associated with the micelle, with the most likely interaction occurring at the micelle/water interface.

TABLE 4: Values of Quantities Extracted from the Hindered Rotor Model

probe	solution	S'[a]	θ_0 (deg)	$D_w \times 10^9$ (s^{-1})	$D_t \times 10^{-9}$ (m^2/s)
AT	aqueous		90	4.07	
	CTAB		90	2.65	
	SDS		90	3.09	
	Thesit		90	3.33	
boc-7AT	aqueous		90	2.65	
	CTAB	0.741	35	0.335	0.456
	SDS		90		
	Thesit		90	2.65	
DD-7AT	aqueous		90	1.70	
	CTAB	0.696	39	0.496	0.761
	SDS	0.768	33	0.858	1.36
	Thesit	0.807	30	0.350	0.640
TRP	aqueous		90	3.27	
	CTAB		90	1.67	
	SDS		90	1.34	
	Thesit		90	3.55	
boc-TRP	aqueous		90	2.10	
	CTAB	0.764	34	0.546	0.760
	SDS		90	1.34	
	Thesit	0.673	40	0.976	3.81
DD-TRP	aqueous		90	1.41	
	CTAB	0.711	38	0.440	0.845
	SDS	0.674	40	0.467	0.381
	Thesit	0.849	26	0.232	1.65
NATA	aqueous		90	2.31	
	CTAB		90	2.35	
	SDS	0.555	48	1.97	1.15
	Thesit		90	2.28	

[a] S' is the order parameter, determined from fits to eq 4. [b] $\theta_0 = \cos^{-1}(0.5(\sqrt{1+8S'} - 1))$. Uncertainty in the determination of S' is taken to be ±5%.

The recovered diffusion coefficients for the probes in water vary inversely with probe size, an expected result. It is also useful to consider the magnitude of D_t, the translational diffusion coefficient along the micelle/water interface. The probes exhibit moderately fast translational diffusion at the micelle/water interfaces. The self-diffusion behavior of the ionic surfactants used in this study has been investigated previously, with values for D_t the lateral diffusion of surfactant molecule in micelles reported on the order of $(0.2-1.5) \times 10^{-10}$ m^2 s^{-1}.[50,51] The translational diffusion constants we recover for the probes used in this study range from $\sim 10^{-9}-10^{-10}$ m^2 s^{-1}, with the smallest value for the oppositely charged probe/micelle systems. The smallest value of D_t we recover is for DD-TRP in SDS. This probe can interact significantly with the nonpolar interior regions of micelles, with the strength of interaction being similar to that of a constituent surfactant molecule. The combination of

strong dispersion interactions with favorable ionic interactions leads to a probe/micelle system that are relatively tightly bound. We note also the relatively slow translational diffusion constants seen for the boc-derivatized probe/CTAB systems, which we attribute to the existence of attractive ionic interactions. The largest translational diffusion constants we measure are for probes in (neutral) Thesit micelles. The order parameter data in Thesit vary considerably depending on probe. For boc-AT a low-order parameter and large D_t value both point to the absence of extensive order in the micelle, a result consistent with the physical picture of Thesit micelles. DD-Trp has the highest order parameter, indicating a restrictive environment immediately surrounding the probe, and also the second fastest translational diffusion in the study. We explain this apparent contradiction by suggesting that the chromophore is indeed in a highly ordered environment, drawn relatively deeply into the Thesit micelle by its aliphatic moiety and with little opposing force due to the probe's overall hydrophobic nature and absence of ionic interactions competing near the surface mantle region of this neutral micelle. As a result, the experimental reorientation time is higher than expected since, in this calculation, it is assumed that the probe is at or near the surface of the micelle. The DD-AT probe yields differing results from that of its TRP analogue. It too has a high order parameter but with much lower translational diffusion constant than DD-TRP. This is a manifestation of the differing physical interactions of these probes induced by the N_7 in the ring of 7AT. While structurally very similar, this subtle difference in the chromophore results in its association with the hydrophilic alcohol terminated, poly(ethylene glycol) portion of the Thesit micelle. The Azaindole chromophore is well-known to hydrogen bond in alcohols and differences in D_t of a factor of 2 have been attributed to its propensity for hydrogen bonding between the chromophore DPP with TX-100 micelles as compared to the structurally similar, non-hydrogen-bonding DMDPP.[52] The experimental data, in conjunction with the model we use, allow the interpretation of our findings based on a balance between ionic and dispersion interactions.

Conclusions

We have studied the steady-state and time-resolved optical response of several indole and azaindole derived probes in water and aqueous micelle solutions. The charge and structure of the probes dictate interactions with micelles. The reorientation dynamics of the probes were consistent with the model of "wobbling-in-a-cone" dynamics with translational diffusion of the probe along the micelle/water interface for SDS and CTAB, and within the mantle of Thesit micelles, along with slower rotation of the micelle in all cases. Our experimental data reveal the ability of ionic interactions to mediate probe/micelle interactions and that van der Waals interactions can overcome these forces. For the case of oppositely charged probe/micelle systems we see the strongest interactions because ionic and van der Waals forces are acting in concert. Probes with structure in common (aliphatic chain) interact with micelles, regardless of probe charge. Our experimental reorientation data indicate transient interactions between the micelle and the probe oligopeptides, with limited structural freedom for the probe molecules in the apparently viscous micellar environment. Taken as a whole, this work demonstrates that there is a clear balance between ionic and dispersion interactions in amphiphilic systems, and that these interactions can be controlled in a straightforward and intuitive manner through the structure of the probe and surfactant.

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