Dynamics of 7-Azatryptophan Derivatives in Micellar Media. Elucidating the Interactions between Peptide Oligomers and Micelles

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Received: December 10, 2001; In Final Form: March 21, 2002

We have investigated the motional and population relaxation dynamics of the nonnatural amino acid 7-azatryptophan (7AT) in free (zwitterionic) form and bonded to polyvaline oligomers in aqueous micelles. We have studied the oligomers in solutions of anionic, cationic, and neutral surfactants above their critical micelle concentrations. The use of these peptide oligomers enables the study of charge and structure on interactions with aqueous micelles. The 7AT fluorescence population decay and reorientation dynamics are monitored in aqueous and micellar solutions as a function of micellar headgroup charge and oligopeptide chain length. The lifetime data on 7AT are discussed in terms of its local environment, and the data point to partitioning of probes into micelles mediated by ionic and dispersion interactions with the micelles. The reorientation dynamics are indicative of persistent 7AT−micelle interactions and can be understood in the context of the hindered rotor model.

Introduction

Understanding the dynamics of intermolecular interactions in solution has proven to be a substantial challenge to the chemistry community over the past several decades. Solution-phase interactions are important because of their central role in mediating a number of phenomena, ranging from organic synthesis to chemical separations and a wide range of biologically important processes. We are interested in understanding the interactions of selected chromophores with entities that exhibit some level of self-organization in solution.[1−5] Such systems include species undergoing crystallization (phase separation), liquid crystalline materials, and micelles, which are the focus of this work. To investigate these micelles, we have synthesized polyvaline oligomers and have attached the nonnatural amino acid 7-azatryptophan (7AT) for use as a chromophore. Our intent is to understand a simplistic mimic of a biomembrane and the interactions that a peptide oligomer will have with such a system.

We use fluorescence spectroscopy to obtain information on 7AT-tagged peptide oligomers in solution. Fluorescence depolarization gives information on restricted rotational motions that proceed on nanosecond and picosecond time scales in these systems.[6] Tryptophan is a fluorescent amino acid, and it possesses a significant fluorescence quantum yield and a strong absorbance near 280 nm.[7,8] Tryptophan is not an ideal optical probe, however. It is characterized by a nonexponential fluorescence population decay, and for many systems, the presence of multiple tryptophan residues can serve to complicate the site specificity of the information garnered from such experiments. Extrinsic fluorescent probes such as dansyl have been used in the study of proteins, but such probes often attach in a nonspecific manner and disrupt native structure. Site-directed mutagenesis can help overcome these limitations by selective removal of tryptophan residues and replacement by a single fluorescent chromophore the spectral properties of which are distinguishable from all other intrinsic species.[9] In recent years, an effort has been made to develop and characterize the nonnatural amino acid 7AT for use as a biological probe.[10−13] This chromophore has been used to study protein structure and dynamics[14−16] because its spectral response is shifted relative to that of tryptophan, its S_1 population decay is characterized by a single exponential over a wide pH range, and the time constant of the decay is sensitive to the immediate environment of the chromophore.[10,16]

A large amount of research has gone into understanding the structure and dynamics of micelles because these labile structures serve as model systems for biological membranes and as a medium in which chemical reactions and excitation transfer studies can be performed, and because of their ability to solubilize proteins and other organic compounds, micelles have found use in chemical separations. Many investigations of micellar systems utilize the fluorescence of (presumably incorporated) dye molecules to provide information on micellization. We report here on our study of the dynamics of small peptide fragments composed of the fluorescent nonnatural amino acid 7AT linked via peptide bond to valine oligomers (Figure 1) in aqueous micelles formed by anionic, cationic, and neutral surfactants (Figure 2). Our experimental data suggest that the polyvaline oligomer interacts significantly with the hydrophobic portion of the micelle, that the 7AT chromophore protrudes from the micellar structure, and that its motional dynamics are mediated by the identity and charge of the micelle constituent polar headgroup. We interpret these results in the context of the hindered rotor model.

Experimental Section

All reagents were obtained from Sigma-Aldrich, Inc. and used as received. *N-t*-boc-7-Azatryptophan was prepared by the procedure described by Petrich[13] using D,L-7-azatryptophan hydrate, BOC-ON [2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile], and triethylamine in water/dioxane solvent.

Synthesis of *N*-7-Azatryptophan−(1−5)Valine Methyl Ester Peptides (7AT−Val_1 through 7AT−Val_5). A series of

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10.1021/jp014462q CCC: $22.00
Published on Web 05/25/2002

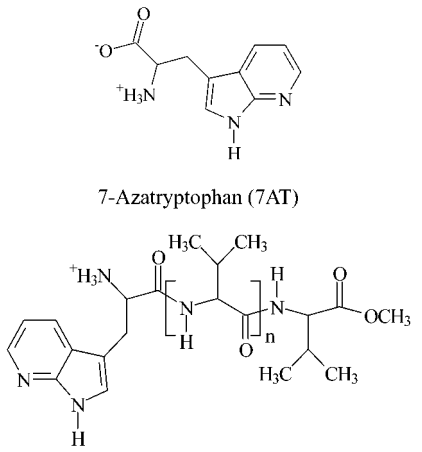

Figure 1. Structures of 7-azatryptophan (7AT) and 7-azatryptophan−valine methyl ester (7AT−Val$_n$), $n = 1-5$.

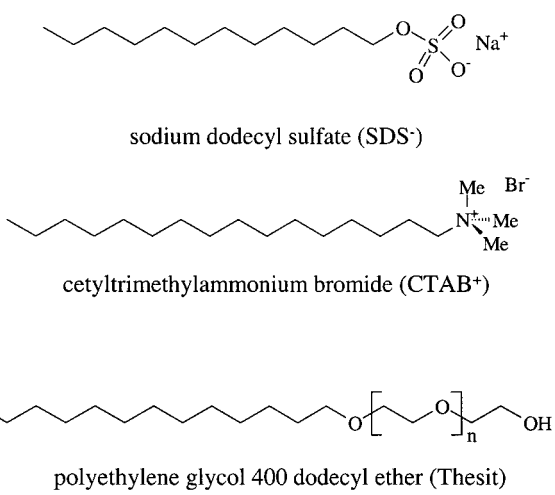

Figure 2. Structure of surfactants used in this work: (top) anionic SDS; (center) cationic CTAB; (bottom) neutral Thesit.

five peptides containing one 7AT and multiple valine (Val) residues was synthesized. In the final form, these peptides all had a free amine *N*-terminus and a methyl ester *C*-terminus. All amino acid couplings were performed using the water-soluble carbodiimide, 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide, hydrochloride (EDCI)[17,18] along with the coupling catalyst hydroxybenzotriazole (HOBt). Throughout the peptide couplings, *t*-boc α-amino protection/deprotection chemistry was used.[19] Peptides were prepared by sequential condensations of *N*-*t*-boc-valine with L-valine methyl ester, followed by addition of *N*-*t*-boc-7-azatryptophan to deprotected and isolated valine peptides. A description of the coupling and deprotection procedure is as follows: EDCI (1.92 g, 10 mmol) and triethylamine (TEA) (1.4 mL, 10 mmol) was added to a stirred solution of *N*-*t*-boc-valine (2.17 g, 10 mmol) in 20 mL of *N*,*N*-dimethylformamide (DMF) at 0 °C. The solution was stirred for 5 min, and HOBt (1.35 g, 10 mmol) was added and stirred for an additional 30 min. In a separate flask, L-valine methyl ester hydrochloride (1.67 g, 10 mmol) and TEA (1.4 mL, 8 mmol) were added to 10 mL of DMF and the mixture was stirred for 5 min, and then filtered to remove triethylamine hydrochloride salt. The filtrate was added to the *N*-*t*-boc-valine vessel, and the mixture was stirred for 1 h at 0 °C and then at room temperature until completion of the reaction as determined by thin-layer chromatography (TLC). The reaction was acidified with aqueous 5% citric acid, and a white precipitate formed. Enough ethyl acetate was added to dissolve and extract the reaction product. The ethyl acetate fraction was washed with citric acid solution, saturated NaCl, and saturated $NaHCO_3$ and again with saturated NaCl solutions, and then dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure to yield product *N*-*t*-boc-valine−valine methyl ester (3.09 g, 66%).

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

surfactant[a]	charge	viscosity (cP)	pH	concentration (mM)/number of cmc	r_h (Å)	τ_M (ns)
CTAB	+	1.00 ± 0.01	6.2	2.5/2.7	25.7	17.6
SDS	−	1.03 ± 0.01	6.6	25.0/3.0	20.7	9.2
Thesit	neutral	0.99 ± 0.01	7.1	1.0/10	35	44.4

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

Deprotection of *t*-Boc-Modified Peptides. A total of 2.5 g of *N*-*t*-boc-valine−valine methyl ester was added to a stirred mixture of 10 mL of trifluoroacetic acid (TFA) and 10 mL of dry dichloromethane (DCM). Thioanisole was added as a scavenger when deprotecting peptides containing 7AT to protect the aromatic ring from attack by liberated carbocations. The progress of the reaction was followed by TLC in $CHCl_3$/MeOH (9:1 v/v). DCM was removed in vacuo, and dry ether was added to precipitate the trifluoroacetate salt. The salt was collected by filtration and washed several times with dry ether. The product was dried over P_2O_5/KOH.

Micelle Preparation. Sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB) (99%+), and Thesit, (poly(ethylene glycol) 400 dodecyl ether) were obtained from Sigma-Aldrich, Inc., checked for fluorescent impurities, and used as received.

Methods. All aqueous solutions were prepared with water from a Milli-Q water filtration system. All solutions used for dynamical measurements were ∼5 μM in 7AT. Upon preparation, surfactant solutions were sonicated for 5 min, stored overnight, and measured the next day. The sample cuvette was temperature-controlled with a brass block holder maintained at 20.0 ± 0.1 °C (Neslab).

To ensure micelle formation, the concentrations of surfactants in this study were several times their critical micelle concentration (cmc). Certain physical parameters and solution properties of the surfactants and micelle solutions must be known for proper interpretation of the data and to ensure that changes in the optical response of the probes are not due to changes in bulk solution properties. The physical parameters of the surfactant solutions used are given in Table 1. One of the relevant physical parameters needed for interpretation of the data is the hydrodynamic radius (r_h) of the micelles. For ionic surfactants, this value is the sum of the micelle core radius, the headgroup radius, and two layers of water.[20,21] For ionic surfactants, this method of determining r_h has been found to match values found from light-scattering experiments. The structure of the nonionic surfactant Thesit is not as well-ordered as CTAB and SDS. The hydrophilic portion of Thesit is made up of a poly(ethylene glycol) chain and not a bulky headgroup. This structural condition produces a hydrated mantle resulting in a less well-defined surface aqueous region, and r_h is estimated at 35 Å.

The azaindole chromophore is sensitive to solution pH because it can be protonated at the N_7 position. This sensitivity is manifested as a significant emission red shift and decreased excited-state lifetime below pH ≈ 5.[22] Thus, the position of the 7AT emission spectrum and the fluorescence lifetime are both sensitive to local environment. Rotational diffusion time

constant(s) are related to "viscosity" according to the Debye−Stokes−Einstein equation and its modifications.[23−25] The viscosities of surfactant solutions were determined by bulk measurements using a viscometer, and the pH was determined using a pH meter, with all measurements being controlled at 20 °C. The small changes in bulk solution properties associated with the formation of the micelles will not affect the optical response of the chromophores substantially. The viscosity changes associated with the addition of amphiphiles to form micelles are negligible, and the pH changes do not occur in a range that affects the protonation of the chromophore or the polyvaline peptides.

Steady-State Absorption and Emission Spectra. Absorption spectra were acquired using Cary model 300 double beam UV−visible absorption spectrometer. Absorption data were collected with 2 nm resolution. Emission spectra were recorded with a Spex Fluorolog 3 spectrometer with 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 that we used for the lifetime and dynamical measurements in this work has been described in detail before.[26] The light pulses used to excite the sample are produced with a synchronously pumped, cavity-dumped dye laser (Coherent 702-2). The dye laser is excited by the second harmonic of the output of a continuous wave (cw) mode-locked Nd:YAG laser (Quantronix 416). Samples were excited with 290 nm light (rhodamine 6G dye, Kodak, KDP Type I SHG). Emission was monitored at the florescence maximum of 7AT in the solution of interest using a 20 nm collection band-pass. Fluorescence was collected at 0°, 54.7°, and 90° with respect to the polarization of the incident pulse. The instrument response function for this system is typically 35 ps fwhm, and lifetimes measured range from ∼600 ps to ∼1.2 ns. We did not need to deconvolute the instrument response function from the experimental data. The shortest reorientation times that we measure are on the order of 50 ps, and because we can recover the entire anisotropy decay, some loss of the initial portion does not affect the accuracy of our determinations. Because of the size of the chromophores used (vide infra), it is not realistic to expect any anisotropy components with time constants less than ∼50 ps.

Results and Discussion

The objectives of these experiments are to understand the optical response of a chromophore incorporated into a peptide fragment as a function of chain length and how these peptides interact with aqueous micelles. The peptide chromophore senses and reports on changes in its local environment. In this study, changes of environment are dictated by intramolecular interactions of 7AT with associated valine residues and by specific interactions of the peptide oligomers with surfactant molecules and micellar structures. The optical response of these probes will be used as a means to detect and understand factors that lead to partitioning and surfactant association of probes in micellar environments. We consider the steady-state and time-resolved spectroscopic data separately.

Steady-State Spectroscopy of 7-Azatryptophan and 7AT−(Val)$_{1-5}$ Methyl Ester. The absorbance spectrum of 7AT in aqueous solution is centered at 290 nm, and the emission maximum is 399 nm both in pure water and in surfactant solutions (Figure 3). When 7AT is linked covalently though its carboxyl functionality by formation of a peptide bond with valine, the absorbance spectrum remains unchanged. The emission maxima of the 7AT−(Val)$_{1-5}$ methyl ester series exhibit a small blue shift of 2−3 nm in pure water as a function

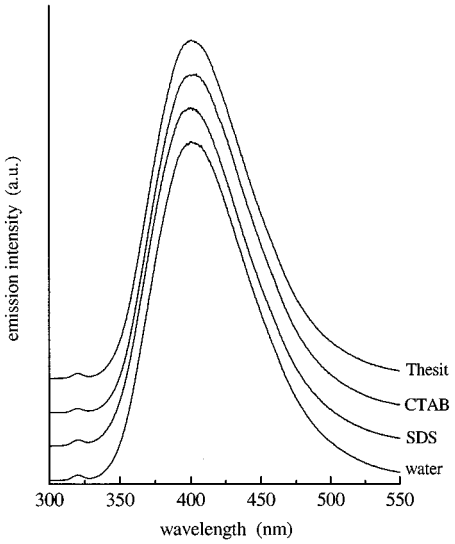

Figure 3. Fluorescence spectra of 7AT in water and surfactant solutions. Spectra have been normalized and are offset for clarity of presentation.

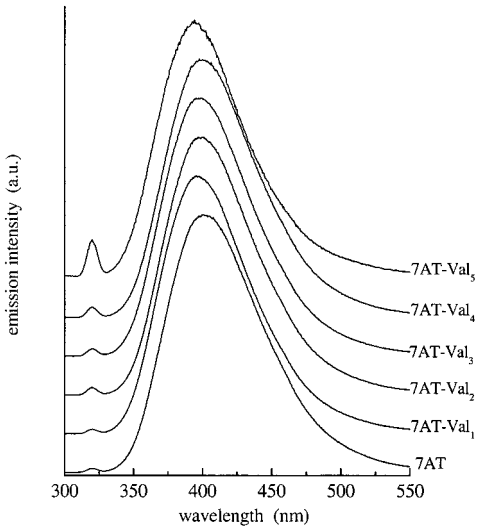

Figure 4. Fluorescence spectra of 7AT and 7AT−Val$_n$ oligopeptides in water. Spectra have been normalized and offset for clarity of presentation.

of the number of valine residues (Figure 4). The 7AT chromophore is known to be sensitive to its immediate environment, which, in this case, is the primary and secondary structure of the peptide to which it is bound. The incorporation of 7AT into peptide fragments is known to cause emission red shifts; the 7AT emission band is centered at 414 nm in Nac-Pro-7AT-Asn-NH_2,[11] 402 nm for Lys−D-7AT−Lys, and 399 nm for Lys−L-7AT−Lys[14] in the pH range of ∼5−8, at which the azaindole chromophore is not protonated. From these studies and the information that we report here, it is clear that the primary structure of a peptide can have a substantial effect on the 7AT emission response and that different enantiomeric forms of a single peptide can cause small shifts in the 7AT emission spectrum. It is not surprising that the 7AT−(Val)$_n$ peptides, differing only in the number of valine residues, exhibit small spectral shifts despite the fact that the chromophore is adjacent to the same residue in all cases. The emission spectra for the peptide series in the cationic surfactant CTAB solution and the neutral surfactant Thesit solution are identical to those in water. We see modest spectral shifts for 7AT in (anionic) SDS solution, but in SDS, the 7AT−(Val)$_{1-5}$ oligomers show a larger blue

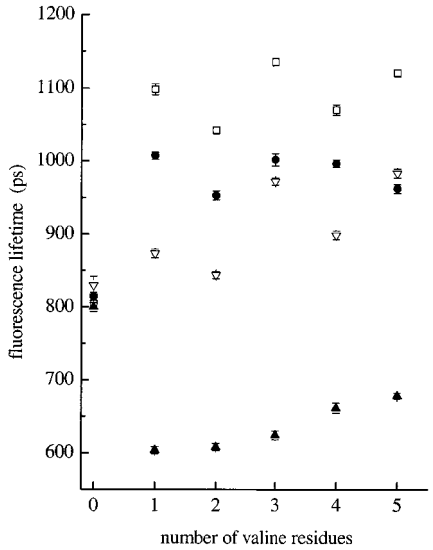

Figure 5. Fluorescence lifetimes of 7AT and 7AT−Val_n ($n = 1-5$) oligomers in (□) water, (●) Thesit, (▽) CTAB, and (▲) SDS. For points at which error bars are not obvious, the uncertainty lies within the vertical dimension of the symbol.

shift of ∼13 nm. Emission shifts of organic dye molecules in surfactant solutions are common and are attributed typically to the dye partitioning into micelles. For example, spectral blue shifts of various indoles have been attributed to incorporation into the micellar core of Brij-35 at concentrations far above the cmc.[27] The steady-state absorption spectra are thus consistent with our expectations and provide some level of insight into 7AT−micelle interactions.

Fluorescence Lifetimes of 7-Azatryptophan and 7AT−$(Val)_{1-5}$ Methyl Ester. Excited-state lifetime measurements can provide information on solution composition and may also contain information on the location of probe relative to the micelle. The fluorescence lifetime of 7AT is sensitive to local environment. 7AT is zwitterionic at pH 7, and its emission 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 generally decays as a single exponential in a nominally uniform environment, with the exception of the octapeptide as reported by Petrich.[28]

In pure water and in micellar solutions, the emission intensity decays of 7AT and all of the AT−Val_n oligomers are best fit to single exponentials, and these decay time constants are shown in Figure 5. Goodness of fit was determined by a χ^2 criterion and visual inspection of residuals of the fit. Single-exponential lifetimes indicate that one fluorescent species is present in a single environment. Upon inspection of the lifetime data, it is obvious that the lifetime of zwitterionic 7AT depends little on the presence of or proximity to micelles. This finding would seem to indicate that 7AT experiences a similar environment in water and micellar solutions and that it does not interact appreciably with surfactant molecules or micelles. When comparing lifetimes of the peptide oligomers in surfactant solutions to those in water, large changes are evident. The largest differences are noted in solutions of SDS. The 7AT peptide oligomer lifetimes decrease significantly in SDS solution. To illustrate this sensitivity, lifetimes were measured for 7AT−Val_1 in solutions of varying SDS concentration (Figure 6). The observed decreases in lifetime with increasing SDS concentration are not the result of simple quenching, nor does the decrease set in only above the cmc. These data show an ∼30% decrease in lifetime to ∼760 ps at a surfactant concentration of 0.5 mM

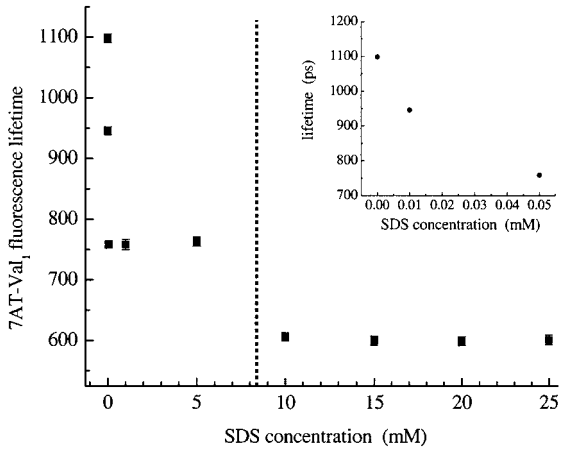

Figure 6. Dependence of 7AT−Val_1 fluorescence lifetime on SDS concentration. The vertical bar indicates the cmc for this system (8.3 mM). The inset shows the dependence of lifetime on low SDS concentration (first three points in the figure).

SDS, followed by a constant lifetime until the cmc is exceeded. Above the cmc, the fluorescence lifetime of 7AT−Val_1 decreases again in a stepwise manner to ∼600 ps. For other micellar solutions, slight oscillations in observed lifetimes were recovered that are analogous to steady-state shifts in emission.

To better understand the nature of interactions between the probe and micelles, we have investigated the rotational diffusion dynamics of our probes in aqueous solutions of cationic, anionic, and neutral micelle-forming surfactants. Rotational diffusion measurements can provide information about intermolecular interactions over the length scale of the probe molecule.[29,30] Reorientation data can distinguish in an unambiguous manner whether probes are experiencing interactions with micelles. Comparing the dynamical response of 7AT and the peptide oligomers in pure water and surfactant solutions will provide direct information on probe−surfactant association.

There is a large body of literature on the rotational diffusion of organic chromophores in single-solvent and binary systems.[30,31] Micellar systems represent a special subset of binary systems, and the issue of reorientation in a micellar environment has been examined before.[32−34] Rotational diffusion has found wide use as a means of probing intermolecular interactions and transient solution-phase organization because the theory is relatively well-established and the relevant molecular interactions proceed on a time scale that can be accessed experimentally. The interactions responsible for determining the reorientation dynamics of a chromophore in solution occur rapidly, and over the course of the chromophore's reorientation, many such intermolecular interactions contribute to the overall experimental observation. A common conclusion of these measurements made in binary solutions is that the molecular-scale interactions responsible for the dynamical behavior of the probes are not dictated by the bulk properties of the solvent. For polar systems, the relevant intermolecular interactions may include dielectric friction,[35] dipolar interactions, and under certain circumstances, formation of solvent−solute complexes. We consider our data on the reorientation of 7AT−Val oligomers in micellar environments. Our measurements point to the strong interaction between the oligopeptide(s) and the micelles, and we interpret our reorientation data in the context of a hindered rotor.

We excite the ensemble of 7AT chromophores in solution with a (vertically) polarized light pulse and monitor the time evolution of emission intensity polarized parallel and perpendicular to the excitation polarization. We combine these data

to generate the induced orientational anisotropy function, $R(t)$,

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

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, and the central issue in such measurements is the application of models and theories to relate the functionality of the $R(t)$ decay to the immediate environment of the chromophore. In principle, $R(t)$ can contain up to five exponential decays, depending on the angle between the transition moments and the shape of the volume swept out by the rotating chromophore. [36] In homogeneous environments, it is unusual to observe more than two decays and the most common case is a single-exponential decay. $R(t)$ decays as a single exponential for 7AT and the 7AT−Val_{1-5} methyl ester peptide series in water. The experimental anisotropy functions for the same chromophore-containing peptides in micellar solutions decay with two exponential components,

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

It is significant that we observe different functionalities for the anisotropy decay in neat solvent (water) and in micellar solutions. This finding points to the incorporation of the probe(s) in micellar environments, and we thus need to consider two different models to account for our experimental findings.

For the case of reorientation in water, a one-component decay is consistent with our earlier findings,[22] and we can interpret these data in the context of the modified Debye−Stokes−Einstein (DSE) model.

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

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

where τ_{OR} is the reorientation time constant, η is the solution bulk viscosity, V is the hydrodynamic volume[37] of the reorienting moiety, 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. The experimental data show a linear dependence of the reorientation time on the number of valine residues (Figure 7), consistent with incremental increases in the hydrodynamic volume of the reorienting moiety. A linear fit of the reorientation times of the positively charged 7AT−$(Val)_{1-5}$ methyl ester series yields a slope of 32.5 ± 1.2 ps/valine. This result is consistent with the predictions of eq 3 in the stick limit, in which the volume of a valine methyl ester moiety is taken to be 123 Å^3 ($S = 1$), yielding a predicted slope of 30 ps/valine.[37] Although these results follow the DSE model, this is not necessarily an expected result. Proteins are known to display segmental motion caused by fast local reorientation of a fluorescent residue and a longer-time component associated with the motion of the peptide as a whole.[38−40] For the short oligomers that we use in this work, segmental motion is not necessarily expected, but it is interesting to note that the data fit well to a single-exponential decay.

We now consider the reorientation of the peptide oligomers in micellar solutions. The first issue to address is whether the chromophore is incorporating in some fashion into the micelle. We assert that the change in functionality of $R(t)$ upon micelle formation indicates incorporation. In cases in which a single-exponential anisotropy decay is seen in micellar solution, the location of the chromophore can be open to question. With the assumption that the oligomers incorporate into micelles, there is an issue of how to model the reorientation of these species. The fluorescence depolarization of a probe molecule in a micelle can be described by up to three independent motions. These motions may include movement of the probe in a restricted region within the micelle, translational motion of the probe as it is adsorbed to the surface of the micelle, and rotation of the micelle as a whole. There are several models that may be applicable to the interpretation of $R(t)$ decays in an environment in which rotation of a species is hindered.[41−44] Two models predict $R(t)$ to decay as a single exponential. In one of these models, the chromophore is attached to the micelle as a rigid entity, with the anisotropy decay time constant representing the motion of the (spherical) micelle as a whole, and we designate this time constant τ_M. The second model that predicts a single-exponential decay of $R(t)$ models the chromophore as being located in the core of a micelle and free to rotate within that confined volume. In this model, the anisotropy is expected to decay as a single exponential with a rate constant of $(\tau_M^{-1} + \tau_R^{-1})^{-1}$, where τ_R is the decay time constant corresponding to chromophore motion in the (homogeneous) core of the micelle. The experimental data suggest that neither of these models are appropriate for the systems that we report on here.

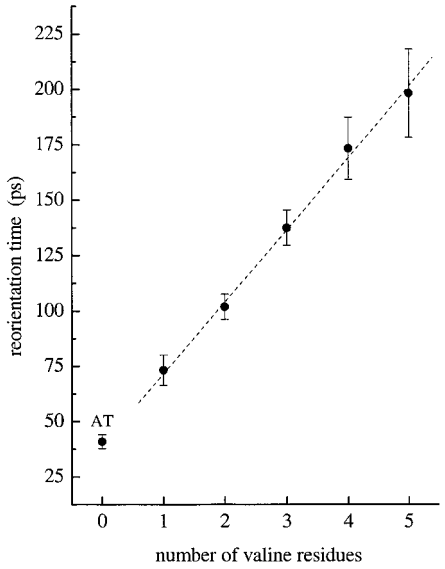

Figure 7. Rotational diffusion time constants of 7AT and 7AT−Val_n as a function of n in water at 20 °C. The slope of the best-fit line for these data is 32.5 ps/valine unit.

There are more complex models that predict anisotropy decays with two or three exponential components, depending on the details of the model. For the case in which the chromophore is bound near the surface of the micelle, a biexponential decay is expected with time constants of τ_w arising from restricted motion analogous to that of a hindered rotor and τ_M for overall reorientation of the cone equal to micellar motion.[42] If translational motion of the chromophore about the surface of the micelle is considered, a third decay component, τ_d, is expected.[32] The anisotropy function for this model is shown in 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 that is a measure of the equilibrium orientational

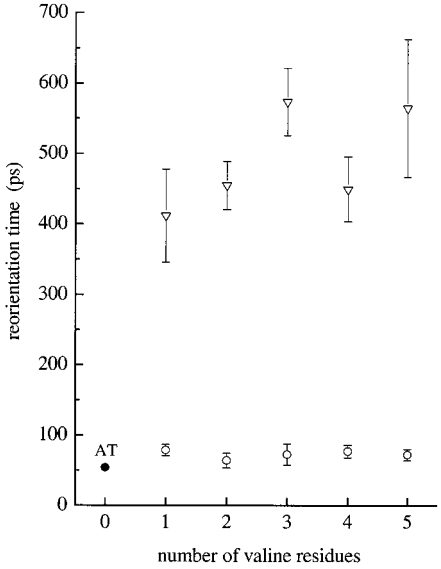

Figure 8. Rotational diffusion time constants of 7AT and 7AT−Val$_n$ in SDS (25 mM, 3 × cmc). The anisotropy functions of the oligomers decay as a two-component exponential, and 7AT decays as a single exponential.

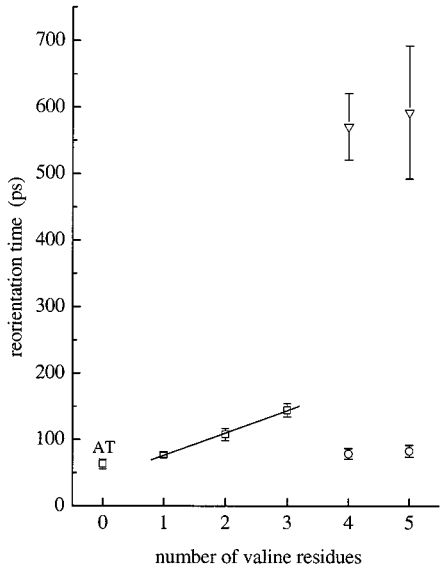

Figure 9. Rotational diffusion time constants of 7AT and 7AT−Val$_n$ in CTAB (2.5 mM, 2.7 × cmc). The anisotropy functions of the oligomers $n = 4$ and 5 decay as a two-component exponential, while 7AT and 7AT−Val$_1$ to 7AT−Val$_3$ decay as a single exponential with a slope of 33.6 ps/valine unit.

distribution of the chromophore transition moment. It is clear from the above discussion that, depending on the complexity of the model used in the interpretation of the experimental data, there is the potential for over-interpretation. We apply Ockham's razor to our treatment of the data.

The experimental data for reorientation of (zwitterionic) 7AT in anionic SDS micellar solution (25 mM, 3× cmc) shows that $R(t)$ decays as a single exponential to within our ability to resolve. The time constant of τ_{OR} of 54 ± 5 ps is measurably longer than that of 7AT in water ($\tau_{OR} = 41 \pm 4$ ps), despite the fact that the bulk viscosity of the SDS solution is essentially the same as that of water. This finding suggests that there is indeed some interaction of the 7AT with the micelle, but the nature of the interaction and its persistence time cannot be addressed directly by these data alone. The reorientation dynamics of the 7AT−(Val)$_n$ oligopeptides in SDS are qualitatively different. For all of the (cationic) oligopeptides in SDS, the experimental $R(t)$ function decays with two exponential components (Figure 8). The average time constants of the two decays are 73 and 491 ps, and there is no clear dependence of these time constants on oligomer length. It is tempting to assume that the interactions of the cationic peptides are mediated by ionic interactions with the anionic SDS micelle headgroups on the basis of a comparison of these data with that for zwitterionic 7AT. On the basis of these findings, it is tempting to presume that the two time constants correspond to fast, possibly restricted motion within the micelle and slower global motion of the micelle as a whole. The difficulty with that interpretation, however, is that neither of the time constants correspond to the expected values for these motions. The most glaring mismatch between experimental data and model prediction is for the longer time constant. The overall motion of SDS micelles is predicted by eq 3 to occur on the several nanosecond time scale. The ∼500 ps decay time constant that we recover appears to be too fast to account for global micelle motion. The peptides do not display single-exponential dynamics or time constants that would be consistent with the probe being bound rigidly in the micelle or free to rotate within the core. Double-exponential decay dynamics could result if the oligopeptides reside in two distinct environments, partitioned between the micelles and aqueous phase of the solution. If this were the case, the short-time components should correspond to the reorientation values in water (Table 3) and the long component should be much longer if it is attached rigidly to or contained within a micelle. The fact that single-exponential fluorescence decays are recovered in some cases supports the notion that the probes are located primarily in a single environment. The anisotropy could yield double-exponential dynamics if the probes are intercalated in the micelle, but the long component recovered for $R(t)$ is much less than the expected τ_M for SDS of ∼9 ns. [34] The slow time constant is not consistent with the motion of the free oligopeptide either (Figure 7), leaving quasi-translational motion of the chromophore along the outer extent of the micelle as the only physical motion consistent with this time constant. We will return to a discussion of this point after considering the reorientation dynamics of the oligopeptides in cationic and neutral micelles.

The reorientation dynamics of the 7AT−valine methyl ester series in (cationic) CTAB surfactant solution (1.0mM, 10× cmc) are shown in Figure 9. The most striking feature contained in these data is the change in reorientation dynamics with increasing oligopeptide length. We observe that the functionality of $R(t)$ is a single-exponential decay in 7AT−Val$_1$ to 7AT−Val$_3$ and $R(t)$ decays as a double exponential for 7AT−Val$_4$ and 7AT−Val$_5$. This behavior is qualitatively different than the dynamics seen in SDS, in which all oligopeptides produced double-exponential anisotropy decays. For the oligopeptides 7AT−Val$_1$ to 7AT−Val$_3$, the slope of the volume dependence for reorientation is 33.6 ± 1.6 ps/valine residue, the same to within the experimental uncertainty as that recovered from the reorientation dynamics for the 7AT−Val$_1$ to 7AT−Val$_5$ oligomers in water. In addition, the reorientation time constants for 7AT−Val$_1$ to 7AT−Val$_3$ peptides in CTAB match those measured in water. These data indicate that the 7AT−Val$_1$ to 7AT−Val$_3$ peptides do not associate with CTAB micelles to a significant extent; they are essentially free in solution. The longer peptides 7AT−Val$_4$ and 7AT−Val$_5$ exhibit a two-component anisotropy decay in CTAB micellar solution with the average time constants for the two components being 76 and 533 ps. It is important to note that the two components are

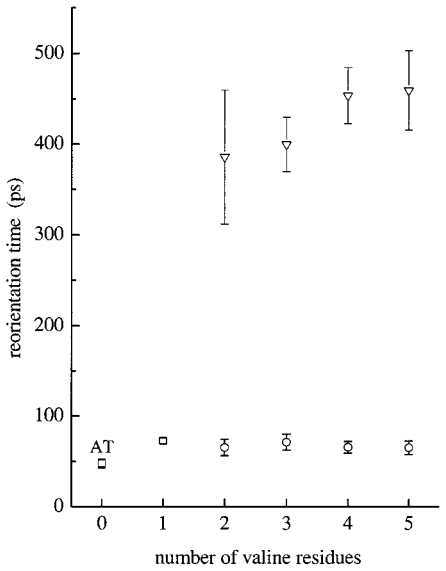

Figure 10. Rotational diffusion time constants of 7AT and 7AT−Val_n in Thesit (1.0 mM, 10 × cmc). The anisotropy functions of the oligomers $n = 2-5$ decay as a two-component exponential, while 7AT and 7AT−Val_1 decay as a single exponential.

characterized by the same time constants for both 7AT−Val_4 and 7AT−Val_5.

It is clear that there is a substantial interaction between the longer oligopeptides and the CTAB micelles, and the dynamics recovered suggest that the interaction is quite similar to that seen for these probes in SDS micelles. We attribute this peptide-length-dependent behavior to the balance between electrostatic and van der Waals interactions in these systems. The cationic oligopeptides will experience ionic (electrostatic) repulsion by the CTAB cationic headgroups, and the strength of this interaction is such that it requires the van der Waals interactions between four or more valine residues and the micelle lipophilic region to overcome electrostatic repulsion. While it is not possible to quantitate the strength of this interaction, we can speculate that the van der Waals interactions will be similar in magnitude to those seen in alkanethiol monolayers on gold, in which the interchain interaction energy is taken to be ~300 cal/mol-CH_2. Clearly, the valine structure is more complicated and the nature of the micellar structure is more fluxional than that of SAMs. For the valine units, there are two CH groups and two CH_3 groups per monomer. If we assume the interaction strength to be the same for these groups, on average, as for CH_2 groups, then four valine residues would produce a strength of interaction roughly equivalent to a C_{16} aliphatic chain or ~4.8 kcal/mol. The corresponding disorder-to-order transition in alkanethiols occurs between 9 and 10 carbons, or 2.7−3 kcal/mol, but these systems are capable of a much greater degree of organization than valine oligomers in micelles. This crude calculation suggests, however, that the electrostatic repulsion term for CTAB−7AT−Val_n interactions is on the order of a few kcal/mol at most.

For the neutral surfactant Thesit, the valine oligomer length dependence of the reorientation dynamics is presented in Figure 10. Not surprisingly, these data present a case that appears to be intermediate between the anionic SDS and cationic CTAB micelle systems. These data show a change in reorientation behavior from single- to double-exponential anisotropy decays for the oligomers, with the onset of a two-component decay beginning at 7AT−Val_2. For Thesit, the dipeptide 7AT−Val_1 displays a single-exponential anisotropy decay with a time constant the same as that measured in water. By analogy to the data for CTAB, the absence of electrostatic contributions to Thesit interactions with the probes reduces the barrier to valine−micelle interactions. Because of the different structures of CTAB and Thesit, the strength of valine−micelle interactions will clearly not be the same for the two systems. For the longer oligopeptides, for which a two-component anisotropy decay is seen, the time constants are independent of peptide length and the slow time constant is slightly smaller than the corresponding data for CTAB and SDS micelles.

We assert that the systems that exhibit two-component anisotropy decays with decay time constants in the ~75 and ~500−600 ps time windows can be explained using a single formalism. The fundamental physics responsible for the data that 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 exhibits only small changes in τ_{OR} for each surfactant solution compared to its behavior in water, indicating that the probe molecule itself does not associate with micelles to a significant extent. This finding is consistent with the lifetime data. (2) Peptides either interact strongly with micelles and manifest a two-component anisotropy decay or do not interact with the micelles to a measurable extent. (3) For systems manifesting a two-component decay, the decay time constants are independent of oligopeptide length in a given micellar system and similar for all three micelles studied here. For this reason, we use the average of fitted decay time constants for a given micelle in the modeling that we discuss below.

Wobbling-in-a-Cone with Translational Diffusion and Micelle Rotation. As discussed above, 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 absence are either that we are not sensitive to such a long-time component, which is not the case, or that the persistence time of the micelle−oligopeptide association is substantially shorter than the reorientation time of the micelle. This explanation is consistent with relatively weak interactions between the probe and micelle, in keeping with our estimate of the interaction energies of several kcal/mol for these systems. We consider that the interaction between the oligopeptide and the micelle structure is characterized by interactions between the lipophilic portion of the micelle and the valine peptide tails and that the 7AT headgroup is in closest proximity to the micelle headgroups, where it is characterized by some freedom to translate or move about on the surface of the micelle. The translational diffusion of a chromophore on a spherical surface will manifest itself as a reorientation of emission dipoles. Equation 4 describes the general case of the anisotropy decay for three independent motions, and we apply the appropriate 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 for τ_M from the DSE equation at 20 °C, or we assume it to be infinitely long, but in either case, we are insensitive to this quantity experimentally. The translational diffusion coefficient may be related to τ_d using the value for r_h (Table 1), the radius of the surface of the micelle over which the probe diffuses, by eq 7.[34]

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

TABLE 2: Values of Quantities Extracted from the "Wobbling-in-a-Cone" Model

surfactant	S'	θ_0 (deg)	$D_w \times 10^9$ (s^{-1})	$D_t \times 10^{-9}$ (m^2/s)
SDS	0.73	36 ± 3	1.2	0.86
CTAB	0.58	46 ± 2	1.7	0.91
Thesit	0.50	52 ± 2	2.3	1.1

[a] θ_0 was calculated according to eq 9. $\theta_0 = \cos^{-1}(0.5(\sqrt{1+8S'} - 1))$. Uncertainty in the determination of S' is taken to be $\pm 5\%$.

TABLE 3: Experimental Diffusion Constants for Oligopeptides in Water

	$D_w \times 10^9$ (s^{-1})
7AT	4.1
7AT−Val_1	2.3
7AT−Val_2	1.6
7AT−Val_3	1.2
7AT−Val_4	0.96
7AT−Val_5	0.83

This diffusion constant is related to the slower of the two motions that we sense. The wobbling-in-a-cone model[42,44] is characterized by a molecule constrained within a cone of semiangle θ_0 with the motion of the molecule in the confined environment being described by a wobbling diffusion coefficient (D_w). We can extract the value of D_w from the experimental data using the quantities τ_w and S',

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

In this model, the quantity θ_0 is related to the order parameter through eq 9.

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

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 2). The values of D_w for all of the peptides in water are given for comparison in Table 3. In water, D_w is calculated using $D_w = (6\tau_{OR})^{-1}$ on the basis of the assumption that in water the order parameter is zero and the equilibrium orientational distribution is random because the probe rotates freely.

The values of the order parameter, S', vary from 0.5 to 0.73 for reorientation of the 7AT−Val_n oligopeptides in the micelles. The order parameter is a measure of the time-averaged orientational distribution of the peptides and can take on values from zero for a fully random orientational distribution to one for completely ordered systems. The values recovered for the order parameter indicate that the peptide oligomers do indeed have a preferential orientation with the micelle surface. In SDS ($\theta_0 = 36°$), they are considerably more confined than in CTAB ($\theta_0 = 46°$) and Thesit ($\theta_0 = 50°$). In SDS, there is clear evidence for order, but the proximity of the cone angle observed in Thesit to the magic angle suggests substantial disorder.

The diffusion coefficients for the peptides in water decrease with peptide size, according to the increase in correlation times (Table 3), and this is an expected result. The recovered parameter D_w for the 7AT−Val_n oligomers demonstrates that the 7AT chromophore is free to reorient about its tethering bond to the valine chain. The wobbling diffusion constant, D_w, reveals substantial confinement of the 7AT chromophore in micellar environments. The value of D_w in micelles is 2−3 times slower than that for 7AT in bulk water. It is important to keep in mind that the free volume accessible to the 7AT chromophore in the micelle is substantially less than that of 7AT in water. We calculate from the cone angles θ_0 that the volume accessible to the 7AT chromophore in SDS is 7% of a full sphere, in CTAB is 9% and in Thesit is 9.4%. When the D_w data are corrected for accessible volume, we recover an effective viscosity of 47 cP for the SDS micelle, 26 cP for the CTAB micelle, and 18 cP for the Thesit micelle. While these viscosities may seem high, they are not substantially different than that of bulk 1-dodecanol or ethylene glycol (∼20 cP). In the glycols, hydrogen bonding is thought to dominate the viscous flow properties, while in the *n*-alcohols, van der Waals interactions are thought to be responsible.

It is also useful to consider the magnitude of D_t. The oligopeptides exhibit moderately fast translational diffusion on the surface of the micelles. The self-diffusion of the ionic surfactants in this study has been investigated previously and is reported to be on the order of $(0.2-1.5) \times 10^{-10}$ m^2 s^{-1}.[45,46] The translational diffusion constants that we recover for the oligopeptides used in this study are $\sim 10^{-9}$ m^2 s^{-1}, a factor of ∼10 faster than surfactant self-diffusion. This is not necessarily a surprising result because of the inability of the oligopeptides to incorporate into the micellar structure with the same efficiency as a constituent surfactant molecule. The largest translational diffusion constant that we measure is for the neutral Thesit micelles. We note that Thesit is the least-ordered micelle used in this study and the intrinsic disorder of the micelle may be related to efficient translational diffusion. The loosely organized hydrophilic ethylene glycol chains create the surface and outer layer of Thesit micelles.

Conclusions

We have studied the steady-state and time-resolved optical response of 7AT and 7AT−Val_n peptide oligomers in water and aqueous micelle solutions. The charge and chain length of the peptides dictate interactions with micelles. The reorientation dynamics of the peptides were consistent with the model of wobbling-in-cone dynamics with translational diffusion of the dye along the surface of the micelle with rotation of the micelle as a whole. Our experimental data reveal a balance between electrostatic or ionic interactions and van der Waals interactions for polyvaline oligopeptides and selected micellar structures. For the case of anionic micelle headgroups and cationic oligopeptides, we see the strongest interactions because ionic and van der Waals forces are both attractive. For the cationic micelle CTAB, we observe the balance between ionic and van der Waals interactions because of the opposing nature of these forces. This balance allows us to estimate the strength of ionic interactions between the probe and the micelle to be on the order of several kcal/mol. Using a neutral surfactant Thesit provides an intermediate case, as expected. 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.

Acknowledgment. We are grateful to the National Science Foundation for support of this work through Grant CHE 0090864.

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