Molecular Dynamics of Guest Molecules in Micelles: Models Based on Fluorescence Polarization Dynamics

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Time-resolved fluorescence polarization (anisotropy) of a probe (guest molecule) in a micelle is used for testing different models of molecular dynamics. The experimental studies so far support the model that includes wobbling motion and translational diffusion for the guest molecule in the micelle.

KEY WORDS: Berry's phase; anisotropy; membrane; soft matter.

INTRODUCTION

The structure of the micelle and the kinetics and photochemistry in micellar solutions have been discussed in several reviews [1–4]. Hydrophobic organic molecules are readily solubilized in aqueous micellar solutions because of the hydrophobic and amphipolar surface/interior region of the micelles. The equilibrium kinetics and other thermodynamic constants of host (micelle)-guest complexes can be understood, in principle, as if the complex is a dynamically varying supermolecule of surfactant assembly and the guest molecule. If the guest molecule is insoluble in water, then it is likely that the molecule is bound to the micelle forever. The noncovalent binding of the molecule in micelle and the dynamically varying micellar shape and size due to surfactant dynamics make the molecular dynamics of the guest molecule in the host micelle a complex one for modeling. The translational and rotational or wobbling dynamics of the guest molecule have important implications for many practical applications of micellar chemistry. However, quantitative estimates of practically useful fluidity parameters such as microviscosity for the micelles require experimental studies using different probes. Theoretical work is still lacking to justify the use of the Stokes–Einstein equation (which is strictly valid only for liquids) for micellar environment. The concept of diffusion of the guest molecule in the restricted space of the micelle vis-à-vis a liquid has to be established by theoretical and experimental studies.

Molecular dynamics of the guest molecule in a host micelle is studied by measuring the time dependence of a property of the guest molecule. The fluorescence property of the molecule is best suited because the polarization of the fluorescence photon carries instantaneous information about the spatial orientation of the molecule [5]. Photon counting fluorescence technology has increased the sensitivity in measurements and dilute samples can be studied.

Since 1978, there have been several pioneering studies which interpreted the nanosecond and picosecond time-resolved fluorescence anisotropy decays to a model of fluorophore dynamics in spherical micelles [6–15]. The early studies [6–9] revealed the power of anisotropy decay data for detailed testing of the models of structure, location, orientation, and dynamics of the probe in the micelle. Better time resolution and enhanced sensitivity in later studies [10–15] were used to support the wobbling-in-cone model of the fluorophore and associated

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diffusive dynamics in the micelle. This article gives an overview of the models for the dynamics of the guest molecule tested in fluorescence polarization studies.

EXPERIMENTAL

Investigation of the dynamics of a guest molecule in a host micelle requires proper experimental conditions. Ideally, there ought to be only one guest molecule in the micelle. The presence of more than one guest molecule in a micelle may lead to guest–guest interaction (quenching or energy transfer), which affects the probing of the guest–micelle dynamics. This condition is achieved experimentally by keeping the ratio of the guest molecule to micelle at 1:*N* where *N*, the number of host micelles for each guest molecule, is very large. If the guest–micelle interaction is sufficiently strong compared to the guest– guest interaction, then the guest molecules will be distributed among micelles according to Poisson's equation:

$$P(n) = \frac{e^{-\frac{1}{N}N^{-n}}}{n!}$$
(1)

where P(n) is the probability that *n* guest molecules are present in one micelle. According to this distribution. The fraction of the guest molecules that are distributed as one per micelle is $e^{-1/N}$, which approaches unity for large *N*. In practice, it is desirable to perform experiments at two or more sufficiently large values of N (>50) and confirm that the values of dynamical parameters are independent of *N*. The surfactant concentration must also be high [far higher than the critical micelle concentration (cmc)] so that the structure of the micelle may be considered stable. Typically, a micelle concentration of 1–20 m*M* and a fluorophore concentration of 1–20 μ *M* are acceptable.

An important criterion for experimental studies is that there is only one type of fluorophore molecule in the micellar solution. If there are two types of fluorescent species (for example, fluorophore in the aqueous phase and micellar phase or different sites of solubilization in the micelle), data analysis of the fluorescence polarization dynamics becomes more complicated [16]. A practical approach to satisfy this criterion is to choose an appropriate excitation and emission wavelength such that the micellar fluorescence is due to only one species. A singleexponential fluorescence decay assures that the dynamics is due to a single species.

Parallel and perpendicular components (F_{\parallel} and F_{\perp}) of fluorescence decays (corrected for the *G*-factor) are obtained by standard experimental procedures in the time

domain [17,18]. The convolution effect and noise are removed by a model-free method [15] to obtain I_{\parallel} and I_{\perp} , which are parallel and perpendicular components for a hypothetical δ -function excitation. The anisotropy decay, normalized to unity, is obtained using Eq. (2).

$$\frac{r(t)}{r(0)} = \frac{I_{\parallel}(t) - I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)} \div \frac{I_{\parallel}(0) - I_{\perp}(0)}{I_{\parallel}(0) + 2I_{\perp}(0)}$$
(2)

The noise-free anisotropy decay can be tested for any of the models of molecular dynamics (see below) in the micelle. In the above approach, knowing the fluorescence decay model is not required. It is also a normal practice to fit F_{\parallel} and F_{\perp} directly using a model of fluorescence decay and a model of anisotropy decay [17].

MODELS OF MOLECULAR DYNAMICS

Experimental results are interpreted in relation to their consistency with one or more models of dynamics of the guest molecule in the micelle. Rotation of the micelle in the aqueous solution is independent of the guest molecular dynamics inside the micelle. The combined effect of the two motions on fluorescence anisotropy decay is

$$\frac{r(t)}{r(0)} = f_a(t) \cdot f_{\rm M}(t) \tag{3}$$

where $f_{\rm M}(t)$ is the decay function due to micelle rotation in the aqueous solution and $f_{\rm a}(t)$ is the decay function due to molecular dynamics in the micelle. The equations for $f_{\rm M}(t)$ and $f_{\rm a}(t)$ are obtained separately.

Micelle Rotation

The decay function due to micelle rotation depends upon the shape and size of the micelle. The decay function is a five exponential for an object of irregular shape [19]. The equation is simple for regular objects. For a spherical micelle of radius a in water,

$$f_{\rm M}(t) = e^{-t/\tau_{\rm M}} \tag{4}$$

$$\tau_{\rm M} = \frac{4\pi a^3 \eta}{3kT} \tag{5}$$

where $\tau_{\rm M}$ is the rotational correlation time of the micelle and η -is the viscosity of the water. For an ellipsoidal micelle, the decay function is two or three exponential, depending upon the location of the molecular axis and transition dipole with respect to the symmetry axes of the ellipsoid [5].

Guest Molecule Dynamics

The guest molecule dynamics in a micelle depends upon various factors such as the site of solubilization, number and distribution of equivalent sites in a micelle, and equilibrium distribution of the orientation of the molecular axis with respect to the surface/interface. These properties depend uniquely on the molecular structure of the guest molecule and surfactant. It is unlikely that there will be a universal model of guest molecule dynamics in the micelle. The practice is to test several models of guest molecule dynamics starting from the simplest one. In choosing a model, one is guided by the guest molecule dynamics in pure liquids of various types and mixed solvents where rotational and translational motion is free. The restrictions in the dynamics when the molecule is confined to the micellar space are then taken into account. The following sequence of experimental protocol is followed.

First, the rotational dynamics of the guest molecule in non-interacting liquids is investigated to establish the molecular size and shape, angle between the absorption and the emission dipoles, and angle between the emission dipole and the molecular axis. For the purpose of investigating guest molecule dynamics in a micelle, one would choose a fluorophore which exhibits ideal behavior in pure liquids. The coincidence of the absorption dipole and emission dipole with the molecular axis and hydrodynamic spherical shape in pure liquids greatly simplifies modeling in micelles. In practice, the fluorophore must show a single-exponential anisotropy decay with an initial anisotropy, r(0) = 0.4, and the rotational correlation time must scale linearly with the viscosity. The restriction of free motion of the fluorophore in the micelle depends upon the nature of interaction between the fluorophore and the surfactant. Three cases are possible.

Case i. The fluorophore is anchored in the micelle in such a way that the molecule does not have independent motion. This is indicated as Model 1 in Fig. 1. Fluorophores with two or more long alkyl chains or covalently linked to a surfactant molecule are examples of this case. The fluorescence depolarization dynamics of the fluorophore is due entirely to the rotation of the micelle:

$$\frac{r(t)}{r(0)} = e^{-t/\tau_{\rm M}}$$
 (6)

Case ii. The structure of the fluorophore is so similar to that of the core region of the micelle that it is solubilized in that region and it tumbles freely (isotropic orientational distribution) as if it is in a liquid of a viscosity comparable to that of the core region. This is shown as Model 2 in Fig. 1. In addition to τ_M , another correlation



Fig. 1. Four simple models of molecular dynamics of a "rod-like" guest molecule in a micelle. The thick line represents the emission transition dipole of the guest molecule. Model 1: The molecule is bound tightly to the micelle. Model 2: The molecule rotates freely in the micelle. Model 3: Molecular rotation is restricted to wobbling in a limited space in the micelle. Model 4: Molecular rotation is restricted but translational diffusion is free.

time, $\tau_r = \eta V/kT$, where η is the viscosity and V is the volume of the fluorophore (spherical approximation), defines the fluorophore dynamics:

$$\frac{r(t)}{r(0)} = e^{-t/\tau_{\rm r}} e^{-t/\tau_{\rm M}}$$
(7)

Case iii. A polar or ionic fluorophore has a preferred site in the interface region where the polarity and other properties vary sharply with distance. A molecule in the interface region may also undergo wobbling about molecular axes and translational motion along the surface. Models 3 and 4 in Fig. 1 belong to this case. The anisotropy

decay function $f_a(t)$ will have to be obtained by an appropriate model of molecular dynamics.

$$\frac{r(t)}{r(0)} = f_{a}(t)e^{-t/\tau_{M}}$$
(8)

Wobbling and Translation of the Guest Molecule and an Equation for $f_a(t)$

In principle, the equation for $f_a(t)$ depends upon the details of the structure, dynamics, and other molecular parameters of the fluorophore-micelle system. When the fluorophore has a preferential orientation in the micelle, then there exists the possibility of wobbling dynamics about the molecular axis. The simplest model wobbling dynamics is the wobbling-in-cone model [20,21]. In this model, the molecular axis is free to wobble within a cone of semiangle θ . The existence of a number of equivalent sites for the molecule in the micelle allows the possibility of hopping from site to site or translational diffusion of the molecule. If one assumes that the equivalent sites are distributed on the surface region of the micelle, then the translational motion may be modeled as two-dimensional diffusion on the surface of the micelle subject to appropriate boundary conditions. An equation for $f_a(t)$ for a specific case was derived in Ref. 15 which is described below.

Consider that the molecular axis of a linear molecule coincides with the emission transition moment and that the molecule is inserted into a spherical micelle. Consider that the molecular axis makes an angle α to the radial direction of the micelle and that the molecular axis wobbles in a cone of semiangle θ_0 . Consider also that the molecule diffuses on the surface of the micelle. That is, the vertex of the wobbling cone diffuses on the surface as shown in Fig. 2. Assuming that the wobbling dynamics and translational diffusion are independent, the anisotropy decay of the molecule due to the two motions is the product of the decay equations derived separately for each motion.

The analytical solution for the decay due to translational motion alone was obtained [15] as

$$\frac{r(t)}{r(0)} = \left(\cos^2\alpha - \frac{1}{2}\sin^2\alpha\right)^2 \exp\left(\frac{-6D_t t}{R^2}\right) + 3\cos^2\alpha \sin^2\alpha \exp\left(\frac{-5D_t t}{R^2}\right) + \frac{3}{4}\sin^4\alpha \exp\left(\frac{-2D_t t}{R^2}\right)$$
(9)



Fig. 2. Same as Model 4 in Fig. 1 with an additional restriction, namely, the emission dipole makes an angle α to the radial direction of the spherical micelle. The molecular wobbling (τ_w) inside the cone of semiangle θ_0 , translational diffusion (τ_t), and rotation of the micelle (τ_m) are shown. It may be noted that τ_t is single valued only for $\alpha = 0$ and not defined for $\alpha \neq 0$ [see Eq. (9)].

where D_t (m² s⁻¹) is the translational diffusion coefficient, *R*(*m*) is the radius of the spherical surface and α is the tilt angle (Fig. 2). The analytical solution for the decay due to the wobbling in cone was derived [20] to be

$$\frac{r(t)}{r(0)} = S^2 + (1 - S^2) \exp(-t/\tau_{\rm w})$$
(10)

where τ_w is the wobbling time constant and *S* is the order parameter. τ_w and *S* depend upon the local structure of the cone and these are related to the cone semiangle θ_0 and wobbling diffusion constant D_w (s⁻¹) as follows [20]:

$$S = \frac{1}{2} \left(1 + \cos \theta_0 \right) \cos \theta_0 \tag{11}$$

$$D_{w}\tau_{w} (1 - S^{2})$$

$$= -x^{2} (1 + x)^{2} \frac{\log\left[\frac{(1 + x)}{2}\right] + \frac{1 - x}{2}}{2(1 - x)}$$

$$+ \frac{(1 - x)(6 + 8x - x^{2} - 12x^{3} - 7x^{4})}{24} \quad (12)$$

where $x = \cos \theta_0$.

For the above model, the anisotropy decay function due to wobbling and translation [$f_a(t)$ in Eq. (8)] is given as the product of Eqs. (9) and (10).

EXPERIMENTAL STUDIES OF FLUORESCENCE DEPOLARIZATION IN MICELLES

Time-resolved fluorescence depolarization of fluorescent molecules intercalated in micelles is a frequently used experimental method for the testing of models of molecular dynamics of a guest molecule in the host micelle. Since the very first experimental study of this kind [7], there have been numerous studies on this topic using different fluorophores in SDS, CTAB, and TX-100 micelles. There is not a single case of guest-host system for which the fluorescence anisotropy was found to be single exponential. This ruled out the possibility of cases i and ii (Models 1 and 2 in Fig. 1), discussed in the previous section. All the molecules studied so far seem to have a preferential orientation on or near the periphery of the surface and the dynamics is constrained. Multiexponential (usually two) anisotropy decays were observed in all cases and these decays were explained by invoking specific model of fluorophore geometry and dynamics.

The model that the fluorophore wobbles about the molecular axis (Model 3 in Fig. 1) together with the tumbling of the micelle was enough to account for the two-exponential anisotropy decay. This model predicts that the higher of the two decay times must be identical to the rotational correlation time of the micelle, which can be independently determined. The expected values are 8.3 ns for SDS, 15.4 ns for CTAB, and 72 ns for TX-100 [14]. The experimental values for several types of the fluorophore in any of these micelles were substantially less than the expected value. Quitevis et al. [12] resolved this discrepancy by adding translational diffusion of the fluorophore to the dynamics. The idea of translational diffusion of a surfactant molecule in a micelle was already used in the analysis of NMR data [22]. The inclusion of translational diffusion improved the analysis and reasonable values were obtained for the diffusion coefficient [12]. It may be noted that the translational diffusion coefficients will have to be multiplied by 2/3 because of an error in the equation for translational diffusion time constant, namely, 4 instead of 6.

Krishna et al. [15] have extended the above model by including the possibility that the orientation of the guest molecule and its emission dipole moment may be tilted by an angle α with respect to the radial direction of the spherical micelle. The equation for anisotropy decay was obtained by Monte Carlo simulation of the diffusion of the tilted dipole in the spherical surface and by exact solution of the diffusion equation. The Monte Carlo simulation method could be used for diffusion on an arbitrary surface as well. It was found that the anisotropy decay due to translational motion of the tilted dipole is single exponential with a time constant of $R^2/6D$ for $\alpha = 0$, two exponentials with time constants $R^2/6D$ and $R^2/2D$ for $\alpha = \pi/2$, and three exponentials with time constants $R^2/6D$, $R^2/5D$, and $R^2/2D$ for $0 < \alpha < \pi/2$. The time constant of $R^2/5D$ is unique in this case because the depolarization contribution arises due to the so-called Berry's phase, a geometrical result common to many other physical problems [23,24].

Krishna et al. [15] used the full equation for the anisotropy decay of a tilted dipole on a spherical micellar surface due to wobbling, translation, and rotation of the micelle to examine the experimental fluorescence anisotropy decay of nilered in SDS micelle. The radius of the micelle was taken to be 16.7 Å [14] and the rotational time constant (τ_m) of SDS micelle was calculated to be 8.3 ns. There remained only four parameters, S, D_{t} , D_{w} , and α , to be determined from the experimental data. It was found that the data of anisotropy decay using experimental fluorescence data with a peak count of 2×10^5 was not sufficient to determine the above four parameters unambiguously. The authors estimated a value of S for nilered in worm-like micellar media at a high concentration of SDS and assumed that this value would be applicable for the spherical micelle as well. The values for $D_{\rm t}, D_{\rm w}$, and α were obtained to be $1.3 \pm 0.1 \times 10^{-10}$ $m^2 s^{-1}$, 2.10 $\pm 0.02 \times 10^8 s^{-1}$, and $1 \pm 2^\circ$, respectively.

Microviscosity in Micelles

One of the motivations for studying the molecular dynamics in micelles is to establish a suitable method for the quantification of the fluidity of the micellar phase. In liquids, the fluidity value is quantified as the shear viscosity, which is measurable by a variety of methods. However, the values of viscosity reported for the micellar phase have been controversial for many reasons [14]. The estimation of viscosity value for the micellar phase is usually done using Stokes–Einstein's equations, which relate translational and rotational diffusion constants to viscosity. For a spherical object of radius a, D_t and D_r (or D_w) are given as

$$D_{\rm t} = \frac{\zeta kT}{6\pi\eta a} \tag{13}$$

$$D_{\rm w} = \frac{\zeta' kT}{8\pi\eta a^3} \tag{14}$$

where ζ and ζ' are constants that are correction factors for the nonspherical shape of the molecule [25]. ζ and ζ' will have to be determined using diffusion data of the molecules in liquids. Thus, one may get an estimate of the fluidity parameter of the micelle if one estimates either one of the above diffusion coefficients for a suitably chosen guest molecule in the micelle. Fluorescence polarization dynamics and the model-based analysis of data is perhaps the only method that promises to deliver both the diffusion coefficients, D_t and D_w , for the guest molecule in a micelle at the concentration level of one per micelle!

The concept that the micelle is an oil drop with an ionic or polar coat and hydrophobic organic molecules are dissolved in the oily interior viscous phase has not been supported by experimental observations. Practically all organic molecules were found to be solubilized in micelles in the interface region [26-28]. Thus, the molecular dynamics of the guest molecule is determined by the physical and chemical properties of the interface region rather than the interior region. This is precisely the region where the physical property (dielectric constant, viscosity, etc.) varies drastically within a distance of a few angstroms. It is not surprising therefore that the values of microviscosity in micelles reported in literature vary widely. The estimated values of microviscosity were found to be probe dependent and technique dependent [9,14,29]. It was found that the $D_{\rm t}$ and $D_{\rm w}$ for the fluorescence probe in micelles give two values of microviscosity for the same probe in the same micelle [13,14]. $D_{\rm t}$ and $D_{\rm w}$ were also found to have different temperature dependencies, contradicting the validity of Eqs. (13) and (14) for micelles. Similar diffusion anomalies called the translation-rotation paradox were reported for organic molecules in polymer solutions near the phase transition temperature [30]. It appears that the validity of Stokes-Einstein equations will have to be justified from first principles for molecules, which are spatially constrained.

SUMMARY

The study of the molecular dynamics of a variety of guest molecules in a host micelle is essential for an understanding of the structure and dynamics of the micelle itself. Fluorescence anisotropy-based time domain methods are best suited for this study; because of their high sensitivity, experiments using low concentrations of the probe (one per micelle) are possible. Analysis of fluorescence anisotropy decay is based on models of guest molecular dynamics. Wobbling motion and translational diffusion of the guest molecule in the micelle are supported by several experimental studies.

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