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Microviscosity of bilayer membranes of some *N*-acylamino acid surfactants determined by fluorescence probe method

Sumita Roy, Ashok Mohanty, Joykrishna Dey *

Department of Chemistry, Indian Institute of Technology, Technology Post Office, Kharagpur, West Bengal 721 302, India

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Abstract

The microviscosities of the self-assemblies formed by sodium *N*-(11-acrylamido-undecanoyl)-aminoacidate, and *N*-(4-*n*-dodecyloxybenzoyl)-aminoacidate surfactants were estimated by fluorescence anisotropy measurements using 1,6-diphenyl-1,3,5-hexatriene as probe molecule. The self-assemblies have high microviscosities in consistence with their bilayer membrane structures. In order to test the validity of the method, the results have been compared with those of micelle forming surfactants, sodium dodecylsulfate, dodecyltrimethylammonium bromide, cetyltrymethylammonium bromide, and Triton X-100 for which the microviscosity data are available in the literature.

1. Introduction

The concept of 'microviscosity' (or more appropriately, microfluidity) for micellar aggregates is relatively new and, therefore, is not well understood. Usually, the polarity and viscosity (or fluidity) within assemblies of amphiphiles are referred to as micropolarity and microviscosity (or microfluidity) since these assemblies are microscopic objects. Unlike Newtonian fluids, there are not many methods to determine microviscosity. However, a considerable effort has been made in the past to measure microviscosity of various self-assemblies formed by synthetic and natural lipids [1–13]. Among various methods, fluorescence probe [2–9], ESR [10–12], and NMR [13] techniques have been widely used by researchers. Since fluorescence probe technique is relatively easy, it remains to be the most popular method. Usually in fluorescence methods, either the intensity ratio of monomer to excimer emission [3–5] of suitable probe molecule or fluorescence anisotropy [2] is used to determine microviscosity. Even though in most of the cases, a very good agreement between the microviscosity values were obtained by these two methods often a large difference between microviscosities determined by use of two different probes using the same technique is reported in the literature [2,4,5]. Since the organized assembly is microheterogeneous in nature, the property of the probe depends on its location. Different probes are preferentially located at different sites and are therefore expected to report different values of the parameter [6]. Nevertheless, microviscosity studies remain meaningful for investigations involving series of homologous surfactants having the same headgroup but of varying alkyl chain length or surfactants with the same headgroup and chain length but of differing chemical structure.

One of the most commonly used fluorescent probes for anisotropy measurement is DPH. DPH is a rather rigid, linear rod-like fluorophore characterized by the

* Corresponding author. Fax: +91 3222 255303.
E-mail address: joydey@chem.iitkgp.ernet.in (J. Dey).

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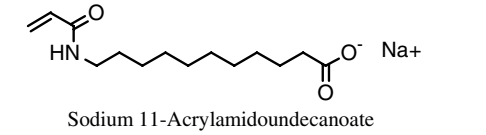
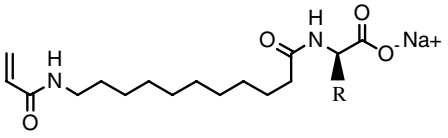
absence of polar groups, and accordingly, it partitions spontaneously into the hydrophobic acyl chain region of lipid bilayers. It has been shown that DPH prefers to locate itself close to the acyl chains, its center of mass being approximately in the middle of the lipid tails and it favors to orient itself approximately in the direction of the membrane normal [14]. Consequently, DPH is used in both static and time-resolved fluorescent techniques [15–20]. The behavior of DPH closely reflects the properties of its local environment in a bilayer. It is commonly used to study both static and dynamic properties of membranes, such as membrane fluidity, ordering of lipid acyl chains, and the location of phase transition boundaries [15,21–23].

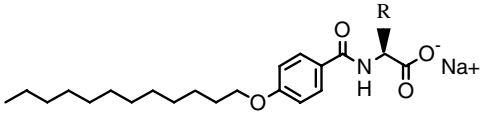
We have recently reported aggregation behavior of two homologous series of *N*-acylamino acid (NAA) surfactants [24–26]. The first consists of sodium *N*-(11-acrylamidoundecanoyl)-glycinate (SAUG), L-alaninate (SAUA), L-valinate (SAUV), L-serinate (SAUS), L-threoninate (SAUT), L-phenylalaninate (SAUPA), L-asparaginate (SAUAS), and L-glutaminate (SAUGL) [25]. The second series of surfactants includes sodium *N*-(4-*n*-dodecyloxybenzoyl)-L-valinate (SDBV), L-leucinate (SDBL), and L-isoleucinate (SDBIL) [26]. The general chemical structures of the surfactants are shown in Fig. 1. These surfactants including sodium 11-acrylamidoundecanoate (SAU) [24] were found to produce bilayer self-assemblies in water having pH above the pK_a (~5.0) of free surfactant molecules. The aim of the present study is to determine microviscosities (η_m) of the bilayer membranes through measurements of steady-state fluorescence anisotropy and lifetime using DPH as a probe molecule. In order to test the validity of the method, we have also determined η_m values for the micelles formed from sodium dodecylsulfate (SDS), dodecyltrimethylammonium bromide (DTAB), cetyltrymethylammonium bromide (CTAB), and Triton X-100 (TX-100) and have compared with those reported in the literature.



Sodium 11-Acrylamidoundecanoate



Sodium N-(11-Acrylamidoundecanoyl)-L-aminoacidate
R= H, CH_3, CH_2Ph, CH_2OH, $CH(CH_3)_2$,$CH(CH_3OH)$, CH_2CONH_2, $CH_2CH_2CONH_2$



Sodium N-(4-n-dodecyloxybenzoyl)-L-aminoacidate
R = $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$

Fig. 1. Molecular structures of SAU, sodium *N*-(11-acrylamidoundecanoyl)-aminoacidate, and *N*-(4-*n*-dodecyloxybenzoyl)-aminoacidate surfactants.

2. Experimental section

2.1. Materials

The fluorescence probe, all *trans*-DPH, was obtained from Aldrich. The probe was purified by repeated recrystallization from acetone/ethanol mixture. The purity was checked by matching absorbance spectrum in methanol with the fluorescence excitation spectra measured at different emission wavelengths. Sodium dodecylsulfate, dodecyltrimethylammonium bromide, cetyltrymethylammonium bromide, and Triton X-100 were obtained from Aldrich and were used after recrystallization either from ethanol or ethanol/acetone mixture. The NAA surfactants were synthesized in the laboratory according to the methods described in the literature [24–26].

2.2. Fluorescence anisotropy measurements

The fluorescence anisotropy measurements were performed with a Perkin–Elmer LS-55 spectrophotometer with a thermostated and magnetically stirred cell housing that allowed temperature control of ±0.1 °C using a Thermo Neslab RTE-7 circulating water bath. The instrument is equipped with a polarization accessory, which uses the L-format instrumental configuration. The steady-state fluorescence anisotropy values (r) were calculated employing the equation,

$$r = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH}), \quad (1)$$

where I_{VV} and I_{VH} are the fluorescence intensities polarized parallel and perpendicular to the excitation light, and G ($= I_{VV}/I_{VH}$) is the instrumental grating factor. The software supplied by the manufacturer automatically determined the correction factor and anisotropy value. In all cases, the anisotropy values were averaged over an integration time of 10 s and maximum number of six measurements for each sample. A 2 mM solution of the probe was prepared in 20% methanol–water mixture. The final concentration of the probe was adjusted to 2 μM by addition of an appropriate amount of the stock solution. Before measurement started, the solution was equilibrated 10 min at 30 °C. The sample was excited at 370 nm and the emission intensity was followed at 450 nm using excitation and emission slits with bandpass of 2.5 and 2.5–5.0 nm, respectively. A 390-nm emission cut-off filter was used to reduce scattered and stray radiation.

2.3. Time-resolved fluorescence measurements

The concentration of the probe in the surfactant solutions was 2.5–3 μM. The solutions were equilibrated for 3 h and then subjected to measurements. All fluorescence lifetime measurements were carried out at ~30 °C. The time-resolved fluorescence intensity decay was measured with a pico-second time-correlated-single-photon-counting (TCSPC) spectrofluorometer. A mode-locked Ti:Sapphire laser (Spectra Physics, Tsunami-3950M 3S) pumped by a diode-pumped CW visible laser (Spectra Physics, Milenia SN 1638) was used as a light source. The frequency doubled output laser ($\lambda = 370$ nm) obtained by a flexible harmonic generator (Spectra Physics, GWU 23 PS) was used for sample excitation. The decay kinetics of DPH was recorded at the emission wavelength of 450 nm. The emission was detected at magic angle (54.7°) polarization using a Hamamatsu MCP photomultiplier tube (2809 U). The instrument response function of the system was ~49 ps. The decay was analyzed by use of IBH DAS-6 decay analysis software. The goodness of the data fit was judged by the χ^2-value (in the range 1–1.2) and by the randomness of residual function.

3. Results and discussion

3.1. Fluorescence anisotropy studies

Fluorescence anisotropy (r) of DPH probe was measured in the presence of surfactants at concentration above the respective critical vesicle concentration (cvc). The measured r-values and the corresponding surfactant concentrations are listed in Table 1. It can be observed that r-values for the micelle forming surfactants are much less compared to bilayer membrane forming surfactants. Slightly higher value of r in TX-100 surfactant is because of its charge neutrality, which facilitates compact packing of the hydrocarbon tails in the self-assemblies. The high value of r in the presence of NAA surfactants, and SAU suggests that the acyl chains are also tightly packed in the aggregates. This means that the fluidity of the hydrophobic core of the self-assemblies is less.

3.2. Fluorescence lifetime of DPH in the presence of surfactants

Fluorescence lifetime of the DPH probe was measured in the presence of surfactants at concentration equal to that at which fluorescence anisotropy was measured. A representative fluorescence intensity decay profile of the DPH probe in SAUA is shown in Fig. 2. In the case of *N*-(4-*n*-dodecyloxybenzoyl)-aminoacidate surfactants, the experimental intensity decay profile fits to monoexponential decay function with χ^2 values in the range 1.0–1.2. That is DPH probe has only one fluorescence lifetime (τ_f) when solubilized in the bilayer assemblies of the above surfactants. This is consistent with the results reported in the literature for DTAB, and TX-100 micelles. However, in the case of *N*-(11-acrylamidoundecanoyl)-aminoacidate surfactants, the decay fits better to a three-exponential decay function with χ^2 values in the range 1.0–1.2. It was observed that two shorter lifetime components of the three-component decays have less contribution (<10%) compared to the major component. For example, in the case of SAUA the three τ_f values were 0.26, 1.65, and 7.05 ns having amplitudes 7.9%, 8.9%, and 83.2%, respectively. The aqueous surfactant

Table 1
Fluorescence anisotropy (r), lifetime (τ_f), rotational correlation time (τ_R), and microviscosity (η_m) of SAU, SAUG, SAUA, SAUPA, SAUS, SAUV, SAUT, SAUAS, SAUGL, SDBV, SDBL, and SDBIL membranes and in TX-100, SDS, DTAB, and CTAB micelles at room temperature (~30 °C)

Surfactant	[Surf] (mM)	r	τ_f (ns)	τ_R (ns)	η_m (MPa s)
SDS	40.0	0.054	6.97	1.22	16.33
DTAB	81.0	0.045	6.97[a]	0.99	13.22
CTAB	5.0	0.058	6.97	1.33	17.77
TX-100	1.5	0.108	7.01[b]	2.98	39.81
SAU	10.0	0.140	6.23	3.93	52.50
SAUG	5.0	0.220	7.25	11.23	150.02
SAUA	5.0	0.180	7.05	6.97	93.11
SAUPA	10.0	0.170	7.20	8.13	108.61
SAUS	10.0	0.140	7.33	4.62	61.72
SAUV	10.0	0.200	7.41	9.15	122.23
SAUT	10.0	0.190	7.65	8.45	112.88
SAUAS	3.5	0.214	7.18	10.38	138.67
SAUGL	5.0	0.186	6.61	6.99	93.38
SDBV	0.125	0.221	6.95	10.89	145.48
SDBL	0.125	0.194	6.82	7.88	105.27
SDBIL	0.125	0.195	7.09	8.28	110.61

[a] From [7].
[b] From [28].

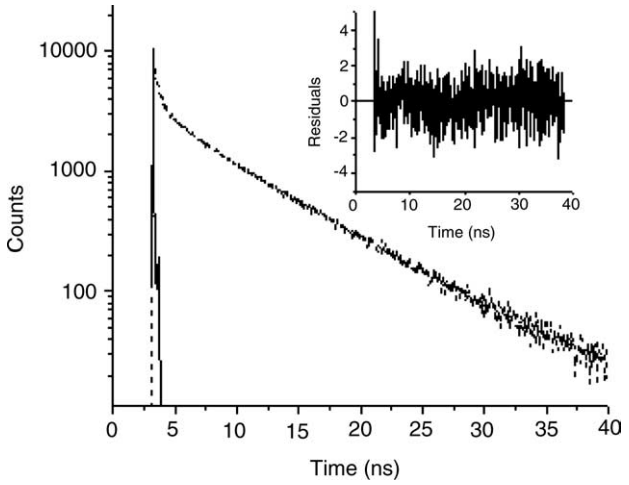


Fig. 2. Fluorescence intensity decay of DPH in 5.0 mM SAUA surfactant; Inset: plot of residual vs. time.

solutions in the absence of any probe did not exhibit any measurable fluorescence intensity at the excitation wavelength of DPH fluorescence. Therefore, the shorter lifetime components were discarded as spurious results, which may have originated from the light scattering by the large vesicular bodies in the solution. Further, the lifetime corresponding to the major component resembles lifetime values measured in the presence of other surfactants as well as in organic solvents of low polarity [27]. The fluorescence lifetime values along with the measured fluorescence anisotropy values of DPH probe in the presence of various surfactants that were used for the calculation of microviscosity of the bilayer membranes are listed in Table 1. The lifetime data of DPH in the presence of DTAB [7], and TX-100 [28] surfactants were obtained from the literature reports. As seen, the fluorescence lifetimes of DPH probe in all the surfactant assemblies are close to each other. Therefore, the fluorescence lifetime values of DPH in the presence of SDS and CTAB surfactants were taken to be equal to that in DTAB micelle. The similarity in the lifetime values suggests that the microenvironments of the probe molecule are closely similar in all the self-assemblies. This means that DPH molecule is mainly solubilized in the hydrocarbon core of the self-assemblies.

3.3. Microviscosity

The microviscosity, η_m, can be calculated from the Debye–Stokes–Einstein relation [29].

$$\eta_m = kT\tau_R/v_h, \tag{2}$$

where v_h is the hydrodynamic volume of the probe molecule and τ_R is rotational correlation time of the fluorophore. The rotational correlation time, τ_R, can be obtained from Perrin's equation [23],

$$\tau_R = \tau_f(r_0/r - 1)^{-1}, \tag{3}$$

where r_0 is the steady-state fluorescence anisotropy in a highly viscous solvent and is taken to be 0.362 [30]. The hydrodynamic volume (313 Å^3) [31] of DPH molecule was estimated from the experimental molecular volume of *trans*-stilbene by employing Edward's atomic increment method [32]. The rotational correlation times and hence microviscosity values thus obtained are presented in Table 1. As can be observed, the microviscosity values of the vesicles formed by the NAA surfactants are in the range 50–150 MPa s. These values are larger compared to the micelle forming DTAB, CTAB, SDS, and TX-100 surfactants. Similar η_m values are reported in the literature for SDS (19 MPa s at 20 °C) [4,33] and CTAB (16.6, 18.8 MPa s at 25 °C) [9] micelles. Therefore, it can be concluded that the η_m values of the bilayer membranes of NAA surfactants studied in this work are accurate within the limit of experimental errors. Slightly higher value of η_m for TX-100 is because of the fact that the surfactant is charge neutral, which results in tighter packing of the hydrophobic tails.

4. Conclusion

It has been shown that DPH is a very good microviscosity probe. The results of the present study demonstrate that the probe is solubilized in the hydrophobic core of the self-assemblies and thus reports the fluidity of this region of the self-assemblies. It can be used to measure microviscosities of molecular self-assemblies having low as well as high values with a fairly good accuracy. Micellar structures formed by SDS, DTAB, CTAB, and TX-100 surfactants have low microviscosities. However, the self-assemblies of *N*-acylamino acid surfactants employed in this work have high microviscosity, which confirms formation of bilayer membranes in aqueous solutions.

Acknowledgements

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[31] The experimental value of the molecular volume (257 $Å^3$) of *trans*-stilbene was taken from [32]. Since this value is 1.45 times of the value obtained by Edward's atomic increment method, the molecular volume (v_h) of DPH probe if calculated by Edward's method would also be ≈1.45 times less than the actual value. In order to obtain a fairly accurate value for v_h, we used the experimentally determined molecular volume of *trans*-stilbene, whose structure is closely similar to that of DPH molecule. The former has an ethylene linkage and the latter molecule has a hexatriene linkage. The volume increment due to extra atoms was calculated by use of the Edward's method. The total increment (38.6 $Å^3$) thus calculated was multiplied by 1.45 and then added to the volume of *trans*-stilbene to obtain v_h (313 $Å^3$) of DPH molecule.
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