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Phospholipid containing mixed micelles Characterization of diheptanoyl phosphatidylcholine (DHPC) and sodium dodecyl sulfate and DHPC and dodecyl trimethylammonium bromide

Radha Ranganathan [a,*], Carolina Vautier-Giongo [a], Mandeep Singh Bakshi [b], Barney L. Bales [a], Joseph Hajdu [c]

[a] *Department of Physics and Astronomy, The Center for Supramolecular Studies, California State University, Northridge, CA 91330-8268, USA*
[b] *Department of Chemistry, Guru Nanak Dev University, Amritsar 143005, Punjab, India*
[c] *Department of Chemistry, The Center for Supramolecular Studies, California State University, Northridge, CA 91330-8268, USA*

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Abstract

Mixed micelles of l,2-diheptanoyl-*sn*-grycero-3-phosphocholine (DHPC) with ionic detergents were prepared to develop well characterized substrates for the study of lipolytic enzymes. The aggregates that formed on mixing DHPC with the anionic surfactant sodium dodecyl sulfate (SDS) and with the positively charged dodecyl trimethylammonium bromide (DTAB) were investigated using time-resolved fluorescence quenching (TRFQ) to determine the aggregation numbers and bimolecular collision rates, and electron spin resonance (ESR) to measure the hydration index and microviscosity of the micelles at the micelle–water interface. Mixed micelles between the phospholipid and each of the detergents formed in all compositions, yielding interfaces with varying charge, hydration, and microviscosity. Both series of micelles were found to be globular up to 0.7 mole fraction of DHPC, while the aggregation numbers varied within the same concentration range of the components less than 15%. Addition of the zwitterionic phospholipid component increased the degree of counterion dissociation as measured by the quenching of the fluorescence of pyrene by the bromide ions bound to DHPC/DTAB micelles, showing that at 0.6 mole fraction of DHPC 80% of the bromide ions are dissociated from the micelles. The interface water concentration decreased significantly on addition of DHPC to each detergent. For combined phospholipid and detergent concentration of 50 mM the interface water concentration decreased, as measured by ESR of the spin-probes, from 38.5 M/L of interface volume in SDS alone to 9 M/L when the phospholipid was

* Corresponding author. Tel.: +1 818 677 2942; fax: +1 818 677 3234.
E-mail address: radha.ranganathan@csun.edu (R. Ranganathan).

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present at 0.7 mole fraction. Similar addition of DHPC to DTAB decreased the interfacial water concentration from 27 M/L to 11 M/L. Determination of the physicochemical parameters of the phospholipid containing mixed micelles here presented are likely to provide important insight into the design of assay systems for kinetic studies of phospholipid metabolizing enzymes.

Keywords: Phospholipids; Diheptanoyl phosphatidylcholine; Mixed micelles; Sodium dodecyl sulfate; Dodecyl trimethylammonium bromide; Hydration; Microviscosity; Aggregation number; Electron spin resonance; Time-resolved fluorescence quenching

1. Introduction

Phosphatidylcholines with two short fatty acyl chains of four to eight carbons each readily form micelles in aqueous solutions (Tausk et al., 1974). They have been widely used as model substrates for the study of lipolytic enzymes (Roberts, 1991; El-Sayed and Roberts, 1985; El-Sayed et al., 1985; Boegeman et al., 2004) as well as for studies of model membranes, including the extraction and reconstitution of membrane-bound proteins (Kessi et al., 1994; Burns and Roberts, 1980). Phospholipid containing mixed micelles and vesicles have been widely used as substrates for measuring the activities of lipid metabolizing enzymes (Boegeman et al., 2004; Cajal et al., 2000; Edwards et al., 2002; Gadd and Biltonen, 2000). Usually, in these assay systems the phospholipid-to-surfactant ratio is varied empirically in order to obtain surface dilution of the phospholipid component, so as to achieve the necessary curve-fitting for interfacial kinetics (El-Sayed et al., 1985; Cajal et al., 2000), without being able to take into account the structure and properties of the micelle lipid–water interface. Specifically, phospholipases have been shown to exhibit several orders of magnitude higher activity toward aggregated substrates versus molecularly dispersed phospholipids in solution (Pieterson et al., 1974; Brzozowski et al., 1991). While the observed catalytic rate enhancements have been attributed to favorable properties of the lipid–water interface (e.g. state of hydration, fluidity, interface charge, surface curvature, conformation of the lipid in the micelle and the micelle-bound enzyme (Tatulian, 2001), the properties of the interface remain to be elucidated. Thus, despite the fundamental importance and widespread application of micelle aggregates in biochemistry, relatively little information is available at the present on the structure and dynamics of these assemblies pertaining to phospholipase enzyme kinetics.

In the present article we report the physicochemical characterization of a series of short-chain phospholipid containing mixed micelles, including diheptanoyl phosphatidylcholine in conjunction with sodium dodecyl sulfate (SDS), and the same phospholipid in mixed micelles with dodecyl trimethylammonium bromide (DTAB), designed to determine the relationship between the properties and composition of these aggregates. The anionic surfactant (SDS) has been selected to provide a simple model for the more complicated, negatively charged bile-salt containing micellar aggregates of phospholipids that serve as substrates of secretory phospholipase A_2 enzymes (Roberts, 1991; El-Sayed and Roberts, 1985), while the cationic series including the DTAB-based mixed micelles are used as reference to obtain a more complete picture of the effect of the charge on the properties of the micellar aggregates.

The short chain lecithin micelles have been studied for their critical micelle concentrations (cmc), size, shape, and structure, by NMR (Burns and Roberts, 1980), Raman spectroscopy (Burns et al., 1982), quasi-elastic light scattering (Burns et al., 1983) and small angle neutron scattering (Lin et al., 1986; Lin et al., 1987). Mixed micelles of ionic/non-ionic or ionic/zwitterionic surfactants yield interfaces of varying charge, hydration, and microviscosity, depending on composition. When well-characterized, they can provide a controlled medium for bio-chemical assays and investigating interfacial phenomena. In the past few years we have applied the techniques of time-resolved fluorescence quenching (TRFQ), electron spin resonance (ESR), and small angle neutron scattering (SANS) to the characterization of micelles and the micelle/water interface (Griffiths et al., 2004; Bales et al., 2001; Ranganathan et al., 2001; Bales and Zana, 2002). This characterization has been framed in the core-shell model, essentially the classical model originally proposed by Hartley (1936). The claim was already made twenty years ago (Halle and Carlström, 1981) that “the overwhelming majority

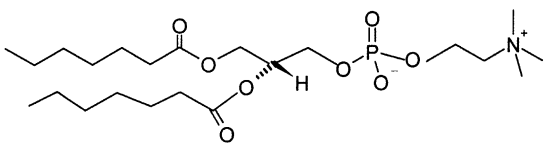
of experimental and theoretical studies have confirmed the classical picture". We consider the basic model to be sound and, in recent work, have proceeded to seek more physico-chemical details of the core-shell model. The core is the oil-like fluid region of the hydrocarbon tails. The shell referred to also as the polar shell, is the micelle/water interface where the enzyme is active. It is worth pointing out that the interface volume of a globular micelle is larger than the core volume. The work reported to date (Ranganathan et al., 2001; Bales and Zana, 2002) involved well studied surfactants affording the opportunity to verify that the new results were in accord with literature results and also provided more insight into the micelle structure. In effect, we have been testing the model at the same time we further characterized those surfactants. In this work, we begin a more ambitious program of studying more complicated systems. Here we investigated two mixed micelle systems of the phospholipid, DHPC, with: (i) sodium dodecyl sulfate and (ii) dodecyl trimethyl ammonium bromide.

The aggregation numbers of DHPC/SDS and DHPC/DTAB micelles and bimolecular collision rates between additive molecules in micelles of various compositions are determined by TRFQ methods. The volume fraction of the polar shell occupied by water and the microviscosity of the micelle/water interface are measured by ESR of spin-probes included in the micelles. The data from the two techniques complement each other, giving a consistent description of the polar shell.

2. Materials and methods

The detergents SDS and DTAB were obtained from SIGMA. The lipid, DHPC (see Scheme 1), was obtained from Avanti Polar Lipids as a lyophilized powder.

TRFQ measurements were carried out with pyrene (Aldrich) as the fluorescence probe. The quenchers used were 3-4 dimethyl benzophenone (DMBP; Aldrich) for DHPC/SDS micelles and cetylpyridinium chloride (C_{16}PC; SIGMA) for DHPC/DTAB micelles. 16-Doxyl stearic acid methyl ester (16DSE; Sigma-Aldrich) was used as the spin probe. All materials were used as received. Nanopure water from Sybron/Barnstead Nanopure II was used as the solvent. The total molar surfactant concentration ([Detergent]+[DHPC]) in all samples studied was 0.050 M. In the samples for TRFQ experiments, the pyrene concentration was kept at about 0.01 of the total micelle concentration and the quencher concentration was roughly 1.5 per micelle (Ranganathan et al., 2000). The spin-probe concentration in the samples for ESR measurements was about 0.25 mM. Measurements were conducted on various mixtures of SDS and DHPC and DTAB and DHPC.



Scheme 1.

2.1. TRFQ

The fluorescence decay curves of pyrene were obtained using an FL900 lifetime measurement spectrometer of Edinburgh Analytical Instruments (EAI). The decay curves were corrected for instrument response. In all aqueous solutions, the dissolved oxygen in water quenches pyrene. In micelles with DTAB the decay rate is further enhanced due to quenching of pyrene fluorescence by the bound bromide ions residing in the polar shell. We exploit this fact to study the concentration of micelle bound Br^- counterions as a function of DHPC molar composition, X_{DHPC}. In order to increase the precision of this study, the oxygen was removed using four freeze–pump–thaw cycles followed by a back filling with argon. The dissolved oxygen does not pose a problem in DHPC/SDS solutions. This was confirmed by conducting TRFQ on a degassed SDS solution with [SDS] = 0.050 M. The pyrene fluorescence lifetime was measured to be 360 ns and the aggregation number derived for the degassed sample was 64, the same as that in the sample before degassing. The pyrene lifetime in DHPC/SDS micelles is typically 180 ns and excellent fits to a micellar quenching decay model are observed. For the determination of aggregation numbers and quenching rates, the corrected curves were fitted to the Infelta–Tachiya model (Infelta et al., 1974; Tachiya, 1975; Gehlen and De Schryver, 1993a) using the Level 2 analysis software of EAI. The details of the experimental set-up and the procedure for recovering the aggregation numbers are described in our previous

publication (Ranganathan et al., 2000). The fits return the values of the average number of quenchers per micelle, A_3, and the quenching rate, k_q. In the absence of quencher migration between micelles within the lifetime of the excited probe, the aggregation number, N, is given by the relation (Gehlen and De Schryver, 1993b),

$$N = \frac{[\text{surfactant}] - [\text{free monomer}]}{[Q]} A_3, \quad (1)$$

where [surfactant] is the total surfactant concentration, which is 0.05 M in all the samples, [free monomer] is the concentration of monomers not in the micelle phase and $[Q]$ is the concentration of quenchers (DMBP or C_{16}PC) in solution. The measurements were carried out at 30 °C. The calculated free monomer concentrations are 4.6 mM in pure 0.050-M SDS solutions and 12.3 mM in pure 0.050-M DTAB (Quina et al., 1995; Ranganathan et al., 1998). The cmc of the mixed systems at various compositions were measured from the ratio of the fluorescence intensities of the first to the third emission peak of pyrene (Giongo et al., 2005; Zana, 1987; Kalyanasundaram and Thomas, 1977). For the mixed systems we take the free monomer concentration to be the cmc. The cmc values are low for ionic/zwitterionic micelles compared to the total detergent concentration of 0.050 M (Giongo et al., 2005) and the uncertainty in N that results from setting [free monomer] = cmc is within the experimental error of ±5%.

2.2. ESR

The polarity sensed by a spin-probe can be detected by measuring its ESR spectrum. The polarity is defined by a hydration index, H, which is the volume fraction of OH dipoles in the spin-probe neighborhood (Griffith et al., 1974; Mukerjee, 1964). Due to their polarity, spin probes are believed to be localized in the micelle/water interface, and sense the water associated with the micelle surface. In other words, H gives a measure of the micelle hydration (Bales et al., 2000a, 2000b). In the core-shell picture (polar shell model) of a micelle, the interface is a shell of headgroups, water, and counterions. Therefore, in the particular case of micelles, H is defined by the volume fraction of water in the polar shell of the micelle, that is

$$H = \frac{V_W}{V_{shell}} = \frac{V_{shell} - V_{dry}}{V_{shell}}, \quad (2)$$

where V_{Shell} is the volume of the polar shell and V_w is the volume occupied by the water in the shell, and V_{dry} is the volume in the polar shell inaccessible to water. In the simple continuum model that has thus far proved successful (Bales et al., 2001; Ranganathan et al., 2001; Bales and Zana, 2002; Bales et al., 2000a, 2000b; Bales et al., 1998; Ranganathan et al., 2003), V_{dry} is the sum of the volumes of the headgroups, the counterions, and any hydrocarbons from the alkyl chains of the surfactants that occupy the shell (Ranganathan et al., 2001).

ESR spectra were taken at X-band using a Bruker ESP 300 E spectrometer equipped with a Bruker variable temperature unit (Model B-VT-2000). The details of sample configuration for ESR have been described previously (Bales et al., 1998). The temperature of the sample was measured with an Omega temperature indicator (model DP41-TC-S2) and was kept constant at 30 °C within ±0.1 °C. Three ESR spectra were acquired for each sample. Fittings of the experimental ESR lines to a Lorentzian–Gaussian sum function were performed using the program LOWFIT. This yields the position of the resonance fields of the three ESR lines with a precision of a few milligauss, and also separates the Lorentzian and Gaussian contributions of the spin label ESR lines. The spacing, A_+, between the low-field and central lines in the ESR is a linear function of the hydration index H as follows (Bales et al., 1998):

$$A_+(H) = 14.210 + 1.552H. \quad (3)$$

Thus, values of H can be found from the nitrogen hyperfine coupling constant A_+. The calibration curve was derived from measurements on water/methanol mixtures at 25 °C. The intrinsic variation of A_+ is only about 5 mG over the range 25–45 °C and Eq. (3) may be used for this range (Bales et al., 2000a, 2000b), The spectral fitting and the stability of the magnetic field gives high precision in A_+ and hence in H.

In addition to H, the ESR linewidths yield the rotational correlation times τ_C and τ_B of the nitroxide group (Bales et al., 1993; Bales and Stenland, 1993). These times are a measure of the rates of rotational motion of the nitroxide group (Schreier et al., 1978) and depend on the viscosity of its environment referred to here as the microviscosity of the polar shell (Bales and Stenland, 1993; Debye, 1929). The rotational correlation times derived from the spectra refer to the rotation of the nitroxide group in the laboratory frame and this

includes both the effects of the rotation of the nitroxide group itself and the rotation of the micelle as a whole. The microviscosity is calculated from the mean rotational correlation time, τ_m, defined as $(\tau_C\tau_B)^{1/2}$, after correcting for micelle rotation (Bales and Stenland, 1993), using the Debye–Stokes–Einstein equation (Debye, 1929; Dote et al., 1981),

$$\tau_m = \frac{4\pi\eta R^3}{3kT}, \quad (4)$$

where η is the microviscosity of the spin label neighborhood, R is the hydrodynamic radius of the spin label (=3.75 Å for 16-DSE) (Bales and Stenland, 1993; Bales et al., 1998), k is the Boltzmann constant and T is the sample temperature.

3. Results

3.1. Time-resolved fluorescence quenching

Excellent fits of the pyrene fluorescence decay to the Infelta–Tachiya micellar quenching model (Gehlen and De Schryver, 1993a) were observed in mixed micelles of DHPC and SDS for $X_{DHPC} \leq 0.7$ and for $X_{DHPC} \leq 0.5$ for DHPC/DTAB. Pure DHPC micelles are known to form large cylindrical micelles of aggregation number >250 (at concentrations of 25 mM and above) at 30 °C with a high degree of polydispersity in the micelle size (Lin et al., 1987). The conditions for the applicability of the Infelta–Tachiya model (Infelta et al., 1974; Gehlen and De Schryver, 1993a) of micellar quenching are not met for DHPC and perhaps for DHPC rich micelles as well. The decay data and fit are shown in Fig. 1 for DHPC/SDS for $X_{DHPC} = 0.7$, the highest DHPC molar concentration for which an Infelta–Tachiya fit could be obtained. Our TRFQ analyses for aggregation numbers and quenching rates are restricted to samples with $X_{DHPC} \leq 0.7$ for DHPC/SDS and $X_{DHPC} \leq 0.5$ for DHPC/DTAB.

3.2. Aggregation numbers

The aggregation numbers according to Eq. (1) are shown in Fig. 2 for DHPC/SDS and DHPC/DTAB micelles at different compositions. There is a rapid rise in the value of N at low values of X_{DHPC} and then a slower decrease to a minimum near $X_{DHPC} = 0.5$ for DHPC/SDS. The overall variation is less than 15%.

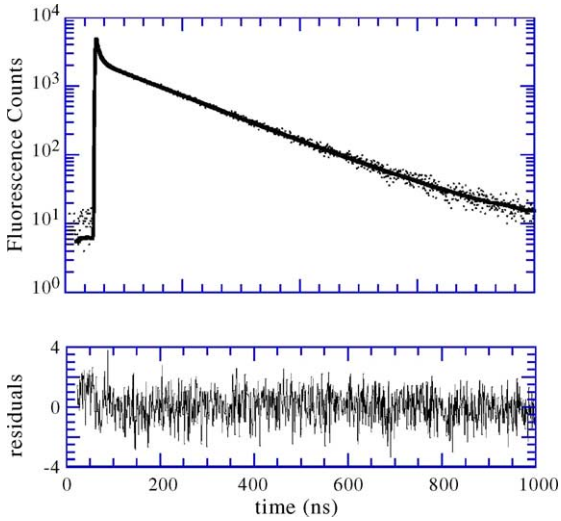


Fig. 1. Fluorescence quenching of pyrene in DHPC/SDS micelles with $X_{DHPC} = 0.7$ due to DMBP quenchers present at an average concentration of 1.1 quencher per micelle. The upper graph shows the data (dots) and fit to the Infelta–Tachiya model for micellar quenching and the lower graph is the residuals of the fit.

3.3. Quenching rates

The diffusion controlled quenching rate, k_q, varies with micelle composition as illustrated in Fig. 3. These rates decrease slightly at low values of $X_{DHPC} = 0$–0.2, where the micelles grow. This decrease is ex-

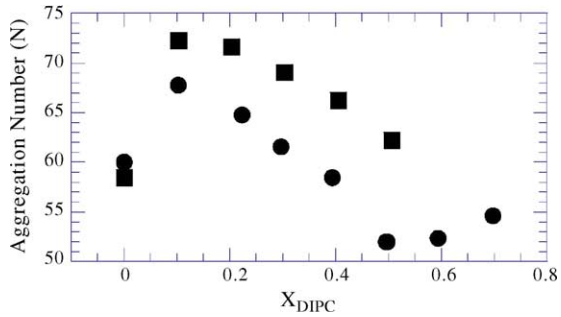


Fig. 2. Aggregation numbers of mixed micelles of DHPC/SDS (●) and DHPC/DTAB, (■) at $T = 30$ °C vs. X_{DHPC}, the molar fraction of DHPC in solution, measured by TRFQ methods, with pyrene probes and DMBP quenchers for DHPC/SDS and C_{16}PC for DHPC/DTAB. The errors in N are ≤±5%.

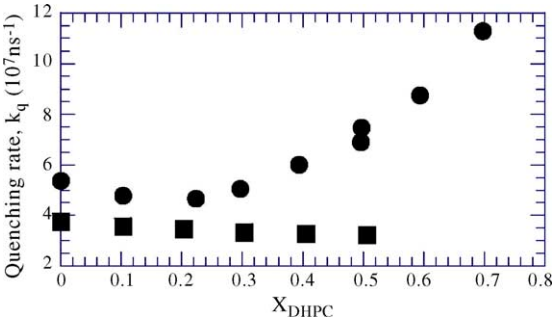


Fig. 3. The fluorescence quenching rate, k_q, in mixed micelles of DHPC/SDS (●) and DHPC/DTAB (■) at $T = 30\,^{\circ}$C vs. X_{DHPC}, the molar fraction of DHPC in solution, measured by TRFQ methods, with pyrene probes and DMBP quenchers for DHPC/SDS and C_{16}PC for DHPC/DTAB.

pected due to the larger volume through which the pyrene-quencher pair must diffuse in order to collide (Ranganathan et al., 2003). Above $X_{DHPC} = 0.1$, k_q increases rather substantially in DHPC/SDS while it continues to decrease slightly in DHPC/DTAB.

3.4. Charge of DHPC/DTAB micelles

The micelle bound bromide ions quench pyrene fluorescence. In the absence of any other quenchers, a single exponential decay of pyrene fluorescence decay with lifetime T_0 is observed, as shown in Fig. 4. The

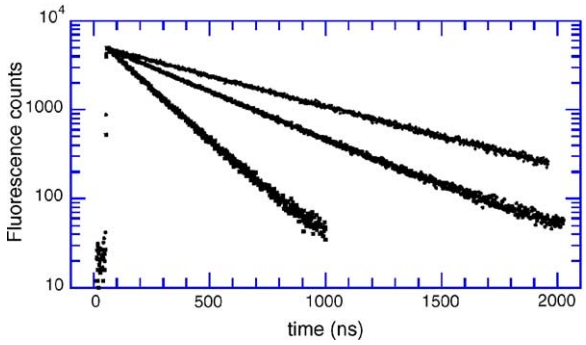


Fig. 4. Fluorescence decay of pyrene in micelles of DTAB (lower curve), in a 50–50 molar ratio of DTAB to DHPC (middle curve) and in DHPC (upper curve) in the absence of any added quenchers. The longest lifetime is in DHPC micelles and the shortest in DTAB micelles. Diffusion controlled quenching of pyrene fluorescence by the micelle bound bromide ions causes the lifetime of pyrene to decrease as the bromide concentration in the interface increases due to mixing of DTAB with DHPC.

shortest T_0 is 180 ns for pure DTAB. T_0 increases as the DHPC composition increases and reaches a value of 390 ns in DHPC. The charge of the mixed micelle is calculated as follows (Zana et al., 1991). The calculation requires the values of the pyrene fluorescence lifetimes under different conditions: T_0 in the degassed (no oxygen) sample, and T_{0DHPC} when there is neither oxygen nor bromide quenching, and T_{0DTAB} in degassed DTAB. Pyrene fluorescence decay in degassed pure DHPC micelles gives T_{0DPHC} and was measured to be 390 ns. These times are related to the concentrations of micelle bound bromide ions ($[Br^-_{mic}]$) because the fluorescence from the micelle solubilized pyrene is quenched by quenchers associated with the micelle. The relations between the lifetimes and bound bromide concentrations are expressed by the rate equations,

$$\frac{1}{T_{0DTAB}} - \frac{1}{T_{0DHPC}} = k_{q0}[Br^-_{mic}]_{DTAB}, \tag{5}$$

$$\frac{1}{T_0} - \frac{1}{T_{0DHPC}} = k_{q0}[Br^-_{mic}], \tag{6}$$

where k_{q0} is the quenching rate constant for quenching of pyrene fluorescence by bromide and is the same for all samples. Any difference in k_{q0} between samples of different compositions due to microviscosity is not significant because of minor variation with composition in this property (Fig. 7). Dividing Eq. (6) by Eq. (5), gives

$$\frac{[Br^-_{mic}]}{[Br^-_{mic}]_{DTAB}} = \frac{(1/T_0) - (1/T_{0DHPC})}{(1/T_{0DTAB}) - (1/T_{0DHPC})} \tag{7}$$

For the determination of micelle charge, the relevant property is the counterion dissociation factor, α, defined as the fraction of the total bromide ions present in solution (=[DTAB]) that is dissociated. Thus,

$$1 - \alpha = \frac{[Br^-_{mic}]}{[Br^-]} = \frac{[Br^-_{mic}]}{[DTAB]}. \tag{8}$$

The quantity [DTAB] is the concentration of the total DTAB in the micelle solution and α is the dissociation factor of the mixed micelle. For pure DTAB micelles, $\alpha = 0.25$ (Bales and Zana, 2002). Therefore, the micelle bound bromide concentration in pure DTAB only micelles from Eq. (8) is,

$$[Br^-_{mic}]_{DTAB} = 0.75[DTAB] = 0.75 \times 0.05. \tag{9}$$

In the second equality, [DTAB] is set equal to 0.05 M, the concentration, before mixing DHPC, used in this investigation. Using Eq. (9) in Eq. (7) gives

$$[\mathrm{Br}^-_{\mathrm{mic}}] = \frac{(1/T_0) - (1/T_{0\mathrm{DHPC}})}{(1/T_{0\mathrm{DTAB}}) - (1/T_{0\mathrm{DHPC}})} 0.75 \times 0.05. \tag{10}$$

The counterion dissociation factor, α, in mixed micelles, using Eq. (10) in Eq. (8) is

$$\alpha = 1 - 0.75 \frac{0.05}{[\mathrm{DTAB}]} \frac{(1/T_0) - (1/T_{0\mathrm{DHPC}})}{(1/T_{0\mathrm{DTAB}}) - (1/T_{0\mathrm{DHPC}})} \tag{11}$$

For the mixed micelle concentration and compositions investigated in this work, [DTAB] in Eq. (11) varies from 0 (in the case of pure DHPC) to 0.050 M (pure DTAB). Values of $1/T_0$ (left-hand ordinate) and that of α for mixed micelles (right-hand ordinate) are graphed in Fig. 5a. The charge of a DHPC/DTAB micelle is given by the number of dodecyl trimethyl ammonium monomers, N_{DTA}, in the micelle and α. For surfactant concentrations well above the cmc, where the micelle composition may be taken to be the same as the solution composition (Bales et al., 2001), the micelle charge of DHPC/DTAB micelles is

$$\text{micelle charge} = e\alpha N_{\mathrm{DTA}} = e\alpha N(1 - X_{\mathrm{DHPC}}) \tag{12}$$

where N is the mixed micelle aggregation number and e is the electronic charge. The surface charge of DHPC/DTAB micelles varies as shown in Fig. 5b.

3.5. Hydration index

The hydration index variation with composition, sensed by 16DSE, derived from ESR spectra for the full range of composition is shown by the left hand ordinate in Fig. 6. In the use of DHPC mixed micelles as substrates for lipid hydrolysis, a relevant property is the interface water concentration, $[\mathrm{H_2O_{micella}}]$ (Gadd and Biltonen, 2000; Rao and Damodaran, 2002). The right-hand ordinate shows the water concentration in the polar shell in concentration units ML^{-1} and is computed as follows:

$$[\mathrm{H_2O_{micelle}}] = \frac{\mathrm{H}}{30 \times 10^{-27} N_0}, \tag{13}$$

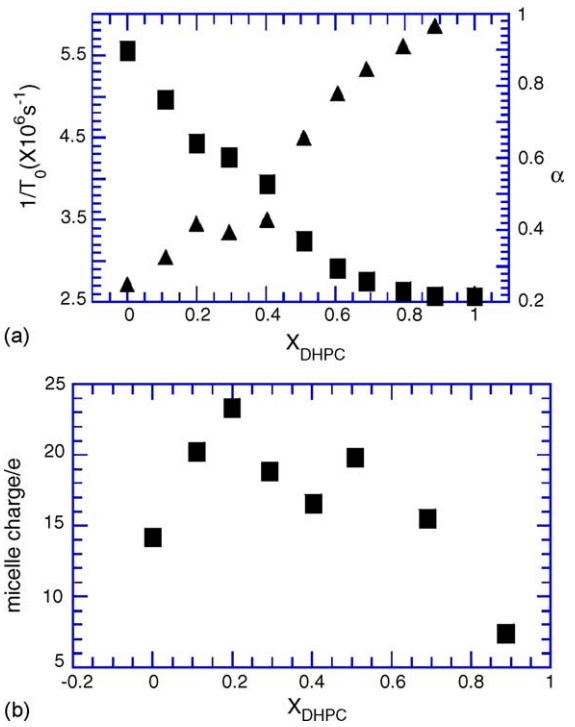


Fig. 5. (a) Pyrene fluorescence decay rates at $T = 30\,^{\circ}\mathrm{C}$ (I/T_0: left side ordinate, ■) decreases as the zwitterionic DHPC component increases due to the release of bromide ions from the interface in mixed micelles. The counterion dissociation factor α (right side ordinate) calculated according to Eq. (11) increases with DHPC. (b) Variation of the charge of mixed micelles of DHPC and DTAB with molar fraction of DHPC, calculated from Eq. (12) using the counterion dissociation factor, α (a) and the measured aggregation numbers (Fig. 2).

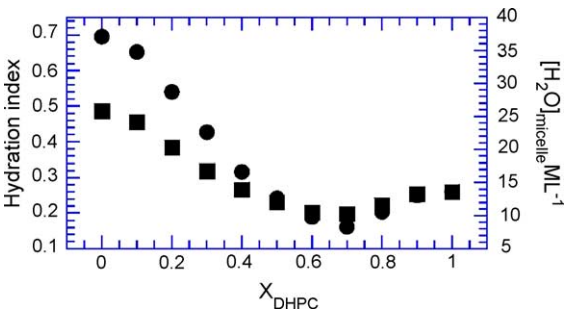


Fig. 6. The hydration index of mixed micelles of DHPC/SDS (●) and DHPC/DTAB (■) at $T = 30\,^{\circ}\mathrm{C}$ vs. X_{DHPC}, the molar fraction of DHPC in solution, measured by ESR in terms of the volume fraction of the interface occupied by water (left side axis). The interface water concentration in units of moles per liter of interface volume (right side axis).

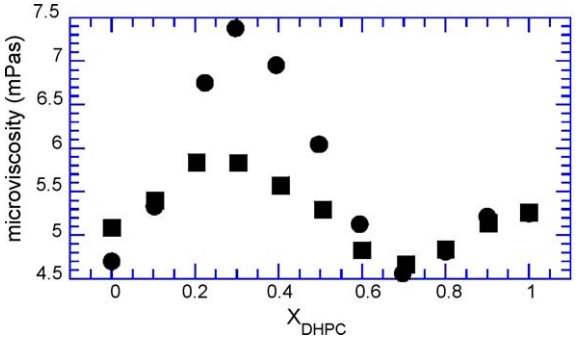


Fig. 7. Dependence of the microviscosity of the polar shell of mixed micelles DHPC/SDS (●) and DHPC/DTAB (■) at $T = 30\,^{\circ}C$ vs. X_{DHPC}, the molar fraction of DHPC in solution. The microviscosity was determined from the rotational correlation time (Eq. (4)) of the spin-probe, 16-DSE.

where the molecular volume of water is 30×10^{-27} l and N_0 is Avogadro's number. Fig. 6 shows the variation in the interface concentration of water as the phospholipid replaces the ionic component in the micelles.

3.6. Microviscosity

The microviscosity data are presented in Fig. 7. After a minor increase with X_{DHPC} up to $X_{DHPC} = 0.3$, the microviscosity decreases to values near that in pure micelles.

4. Discussion

4.1. Polar shell model

In studies of surfactant and salt induced growth of several ionic micelles, the hydration index is found to decrease linearly with increasing aggregation number up until a certain aggregation number depending on the type of surfactant (Bales et al., 1998). The observed decrease and linearity are consistent with the polar shell model calculations for a spherical geometry. The number of water molecules per headgroup decreases as the aggregation number increases suggesting that the water associated with the micelle is displaced as the growing numbers of headgroups are accommodated (Bales et al., 1998). The hydration index and aggregation number are thus related properties. For definite globular shapes of spheroids or ellipsoids, the relation may be calculated by taking the polar shell volume, V_{Shell}, to be the sum of the volumes occupied by headgroups, counterions, water and some hydrocarbon (Ranganathan et al., 2001; Bales and Zana, 2002). The polar shell model clearly shows that a continuous decrease of hydration index accompanying growth is an indication that micelles remain globular. The polar shell view also helps us understand why the hydration remains constant after SDS micelles transform into cylinders. When the aqueous sodium ion concentration increases beyond 0.4 M, micelles start to grow rapidly and H reaches a constant value (Bales et al., 1998). The transformation from a slow growth, decreasing H to a rapid growth, constant H was consistent with a change in shape from spherical to cylindrical micelles (Ranganathan et al., 2001; Bales et al., 2000b). When the constraint of a spherical shape is removed, micelles can grow rapidly lengthwise, with a constant N/ℓ, where ℓ is the length of the cylinder. If the radius, r, of the cylinder and hence the surface area per headgroup, given by $N/(2\pi r\ell)$, are constant as well then the number of water molecules per headgroup will be constant. This results in a hydration index that is constant in the cylindrical growth region as observed for pure SDS micelles. The changes in the hydration index and aggregation number can be interpreted together for the present system of mixed micelles as well using the polar shell view as discussed below.

4.2. Aggregation number (Fig. 2)

The limited variation of about 15% in the aggregation number with composition for SDS/DHPC and DTAB/DHPC micelles and the continuous decrease in H are indicative that the mixed micelles remain globular at compositions of $X_{DHPC} \leq 0.7$. Cylindrical micelles generally tend to grow rapidly in size along the length (Ranganathan et al., 2001). Fluorescence decay due to quenching in cylindrical or disc-like micelles do not give reasonable fits to the Infelta model (Swanson-Vethamuthu et al., 1996).

4.3. Hydration index (Fig. 6)

The bromide ion and the DTA^+ headgroup being larger displace more water from the polar shell than the smaller sodium ion and the sulfate headgroup of SDS. This could result in the hydration index of pure DTAB being less than that of pure SDS while the aggregation number remains the same. Displacement of water in order to accommodate the larger DHPC head-

groups may account for the drop in H upon addition of DHPC to either ionic micelle. Furthermore, inclusion of non-ionic or zwitterionic components into ionic micelles is expected to reduce the surface charge density and hence the concentration of micelle bound counterions (Moya and Schulz, 1999; Hayter, 1992). With a decreased concentration of bound counterions there is no more of the required counterion-bound hydration water and H continues to decrease. As may be noted from Fig. 5a for DHPC/DTAB, the counterion dissociation factor increases with X_{DHPC}. The hydration index values are the same for the DHPC/SDS and the DTAB/DHPC micelles when $X_{\mathrm{DHPC}} > 0.5$. The decrease in the hydration index or the interface water concentration upon mixing DHPC into the ionic micelles is quite significant.

The TRFQ data for $X_{\mathrm{DHPC}} > 0.7$ could not be suitably fit to the Infelta Model. $X_{\mathrm{DHPC}} = 0.7$ is also the composition at which H changes behavior from continuously decreasing to slightly increasing. Formation of large cylindrical polydisperse micelles could account for the changes in the growth and hydration pattern. In large cylindrical micelles, the probe-quencher encounter probability within the excited state lifetime of the probe is lowered because of the large available volume for diffusion (Almgren, 1991; Swanson-Vethamuthu et al., 1996). Polydisperse micelles lead to a distribution in k_{q}. These conditions render the data unsuitable for micellar quenching decay analysis using the Infelta model. In the case of the mixed micelles of the present study, upon transformation to cylindrical shapes at X_{DHPC} of about 0.7, the radius of the cylinder may decrease with increasing DHPC, because of the shorter chain length of DHPC. For a cylinder of length ℓ, an increase in the area per headgroup, given by $N/(2\pi r\ell)$ is possible if N/ℓ is constant. For DHPC micelles N/ℓ was found to be constant in SANS measurements (Lin et al., 1987). A decrease in r and the consequent increase in the area per headgroup, $N/(2\pi r\ell)$, could account for the slight increase in H as X_{DHPC} increases beyond 0.7.

4.4. Charge of mixed micelles (Figs. 4 and 5)

Counterion condensation on to the micelle and the resulting charge of the micelle depends on the micelle surface charge density before condensation. With increasing amounts of DHPC in DHPC/DTAB micelles, the micelle surface charge density changes because of the zwitterionic nature of the DHPC headgroup. The observed increase in the fluorescence lifetime of pyrene with X_{DHPC} implies that the bromide concentration in the interface decreases. The counterion dissociation constant, α, estimated from T_0 (Eq. (11)) show that more than about 80% of the counterions are dissociated in DHPC/DTAB micelles when $X_{\mathrm{DHPC}} > 0.6$.

4.5. Microviscosity (Fig. 7)

No significant variation is found in microviscosity. The variation is less than a factor of two. However it is remarkable that this small variation can be detected through the present line-shape analyses. The polar shell microviscosity exhibits a maximum at $X_{\mathrm{DHPC}} \approx 0.3$. Occurrence of a maximum is contrary to the observed monotonic behavior in mixed micelles of SDS and a non-ionic detergent with a sugar headgroup (dodecylmalono-bis-*N*-methylglucamide, DBNMG) that was previously investigated (Bales et al., 2001). Rather a similarity to bulk alcohol/water mixtures may be noted. In ethanol/water mixtures the microviscosity is maximum at 50% ethanol and the hydration index (calculated from composition) decreases continuously from a value of unity for water as ethanol is added to water (Hodgman et al., 1960–1961).

4.6. Bimolecular collision rates (Fig. 3)

Recent work has shown that bimolecular collision rates in SDS micelles, determined by fluorescence quenching, are described by a hydrodynamic model (Ranganathan et al., 2003). Diffusion controlled quenching of pyrene by quenchers is well described by the Stokes–Einstein–Debye equation for the quenching rate k_{q} (Ranganathan et al., 2003),

$$k_q = P\frac{8\mathrm{RT}}{3000\eta}\frac{10^{27}}{N_0 V_{\mathrm{shell}}}, \tag{14}$$

where R is the universal gas constant, T is the sample temperature, N_0 is the Avogadro number, V_{Shell} is the volume in liters of the polar shell whose size depends directly on the aggregation number, and P is the probability that a collision results in quenching (Ranganathan et al., 2003). In mixed micelles of SDS and DBNMG the microviscosity η increases with

the total molar fraction of DBNMG reporting an increase of a factor five from SDS to DBNMG (Bales et al., 2001). The microviscosity in this mixed micelle system largely accounts for the observed variation of a factor six to seven in k_q, because the change in micelle aggregation number was not significant (Bales et al., 2001). In the present mixed micelles of phospholipids and the ionic detergents, the variation of k_q in DHPC/SDS is only about a factor two to three but is stronger than in DHPC/DTAB. The aggregation number and microviscosity variations are also stronger in the DHPC/SDS system than in DHPC/DTAB. Both microviscosity and size (Eq. (14)) can account for the variation in k_q. The slight increase in η and slight decrease in N (for $X_{DHPC} > 0.1$) in DHPC/DTAB contribute towards an almost constant k_q in this system.

5. Conclusions

The biochemical significance of the results here reported is in providing a series of phospholipid containing mixed micelles, characterized by a number of key physicochemical parameters (i.e., size, charge, state of interface hydration, and microviscosity), as a function of micellar composition toward development of well-defined substrates for the study of phospholipid metabolizing enzymes.

Diheptanoyl phosphatidylcholine forms micelles with SDS in all compositions, and up to 0.7 phospholipid mole fraction the globular micelles that form do not grow significantly in size with variation of composition. Counterion dissociation of SDS is promoted with increase of micellar phospholipid content, and the interface water concentration is substantially decreased with increase in phospholipid concentration. Since it has been reported that hydration of the lipid–water interface is an important parameter in modulating micelle-bound enzyme activity (Gadd and Biltonen, 2000; Tatulian, 2001; Rao and Damodaran, 2002), it is expected that the results from our work will provide useful leads toward the design and development of phospholipase substrates with targeted physicochemical properties. The mixed micelle aggregation numbers, bimolecular collision rates in mixed micelles and the micelle/water interface properties of hydration and microviscosity were measured. These micelle properties show a stronger variation with X_{DHPC} for DHPC/SDS than for DHPC/DTAB. The marked change in behavior at $X_{DHPC} = 0.7$ is attributed to a possible change in shape. Micelles do not grow to large sizes until about $X_{DHPC} = 0.7$. The growth/hydration pattern of mixed micelles of DHPC with SDS and DHPC with DTAB are consistent with a change in shape from globular to cylindrical micelles at a DHPC composition of about 70%, with globular shapes prevailing at the lower DHPC concentrations. The variation of H may thus be used to indicate changes in shape or surface curvature of the micelle. Changes in shape are more directly observable by other experimental techniques like Viscosity or Scattering. Counterion dissociation increases as the DHPC is mixed with the ionic component and micelle charge varies reflecting the variation in counterion dissociation factor together with aggregation number and composition. The interface water concentration decreases with inclusion of the phospholipid. The decrease in interface water concentration with increasing phospholipid component is quite considerable. If interfacial water activity is indeed important for enzyme activity, then surfactant concentration and composition become quite relevant parameters in designing substrates.

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