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Short communication

Dipolar rearrangement during micellization explored using a potential-sensitive fluorescent probe

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ABSTRACT

Dipole potential is the potential difference within the membrane bilayer, which originates due to the nonrandom arrangement of lipid dipoles and water molecules at the membrane interface. Although dipole potential is generally used in the context of bilayer membranes, the nonrandom arrangement of amphiphiles and water dipoles would also contribute to dipole potential in organized molecular assemblies such as micelles. In this work, we show that the process of micelle formation from monomers for a representative variety of detergents is associated with dipolar rearrangement. We monitor the dipolar reorganization upon micellization as a change in dipole potential, measured by the dual wavelength ratiometric approach utilizing the potential-sensitive membrane probe di-8-ANEPPS. We further utilized this phenomenon to estimate the critical micelle concentration (CMC) of a variety of detergents. CMC determined by this method are in overall agreement with the literature values of CMC for these detergents. To the best of our knowledge, these results constitute the first report showing dipolar reorientation during micellization. We conclude that dipole potential measurements could provide a novel approach to explore micellar organization.

1. Introduction

Detergents are soluble amphiphiles characterized by a higher degree of hydrophilicity than most double chained phospholipids found in biological membranes (Neugebauer, 1990). They self associate to form thermodynamically stable, non-covalent aggregates called micelles above a critical concentration (strictly speaking, a narrow concentration range), referred to as the critical micelle concentration (CMC) (Tanford, 1978). The general principle underlying micelle formation (*i.e.*, the hydrophobic effect) serves as a common mechanism responsible for formation of organized molecular assemblies such as membrane bilayers. Micelles are highly cooperative and dynamic, and easier to manipulate experimentally (Chaudhuri et al., 2009). They are popularly used as membrane-mimetic media to characterize membrane proteins and peptides (Sham et al., 2003; Raghuraman and Chattopadhyay, 2004; Rawat et al., 2005). Interestingly, the concept of micellization is relevant in the context of solubilization and reconstitution of membrane proteins (Kalipatnapu and Chattopadhyay, 2005; Chattopadhyay et al., 2015). There is a certain correlation between micelle formation and detergent concentration necessary for solubilization (Rivnay and Metzger, 1982).

Dipole potential represents an important and useful electrostatic property of biological membranes. It is the potential difference within the membrane bilayer and is generated due to the nonrandom orientation of electric dipoles of lipid and water molecules at the membrane interface (Brockman, 1994; Clarke, 2001; O'Shea, 2005; Wang, 2012). Dipole potential is generally operative over a relatively small distance and therefore the electric field generated due to dipole potential could be very large ($\sim 10^8$–10^9 Vm^{-1}) (Clarke, 2001; Wang, 2012). Membrane dipole potential has been reported to be a sensitive indicator of the function of membrane proteins and peptides (Duffin et al., 2003; Starke-Peterkovic et al., 2005; Starke-Peterkovic and Clarke, 2009; Singh et al., 2013; Richens et al., 2015) and is often used to monitor the binding of proteins to membranes (Cladera and O'Shea, 1998; Chaudhuri and Chattopadhyay, 2014). Interestingly, membrane cholesterol has been shown to increase dipole potential in model and natural membranes (Starke-Peterkovic et al., 2006; Haldar et al., 2012; Singh et al., 2013) in a stereo-specific manner (Bandari et al., 2014).

The concept of dipole potential is generally applied to membranes although the general scenario for such a concept

Abbreviations: CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; CMC, critical micelle concentration; CTAB, cetyltrimethylammonium bromide; di-8-ANEPPS, 4-(2-(6-(dioctylamino)-2-naphthalenyl)ethenyl)-1-(3-sulfopropyl)-pyridinium inner salt; SDS, sodium dodecyl sulfate.

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would be valid for micelles and similar organized molecular assemblies. In this paper, we show that the process of micelle formation from detergent monomers is accompanied by a characteristic dipolar rearrangement, manifested by a change in dipole potential. This phenomenon could be exploited to evaluate the CMC of detergents monitored by the dual wavelength ratiometric approach utilizing a potential-sensitive membrane probe. These results provide novel insight into dipolar rearrangements that take place during micellization.

2. Materials and methods

2.1. Materials

CHAPS, NaCl and Triton X-100 were obtained from Sigma Chemical Co. (St. Louis, MO). CTAB was purchased from Serva (Heidelberg, Germany). SDS was from Calbiochem (San Diego, CA). Di-8-ANEPPS was purchased from Molecular Probes/Invitrogen (Eugene, OR). All other chemicals used were of the highest available purity. Water was purified through a Millipore (Bedford, MA) Milli-Q system and used throughout.

2.2. Methods

2.2.1. Sample preparation

Various amounts of detergent dispersed in a total volume of 2 ml were prepared in aqueous solution. In order to incorporate di-8-ANEPPS into micelles, a small aliquot containing 1 nmol of di-8-ANEPPS from a methanolic stock solution was added to 2 ml of sample (containing varying amounts of detergents) and mixed well by vortexing for 1 min. The resultant di-8-ANEPPS concentration was 0.5 μM in all cases and methanol content was always low (0.5% v/v). The concentration of stock solution of di-8-ANEPPS in methanol was estimated from its molar extinction coefficient (ε) of 37,000 $M^{-1}\,cm^{-1}$ at 498 nm (Le Goff et al., 2007). Background samples were prepared the same way except that di-8-ANEPPS was not added to them. Samples were incubated in dark for 1 h at room temperature (~23 °C) for equilibration before measuring fluorescence. Experiments were performed with at least three sets of samples at room temperature (~23 °C).

2.2.2. Measurement of potential-sensitive fluorescence intensity ratio

Measurements were carried out by dual wavelength ratiometric approach using the voltage-sensitive fluorescence probe di-8-ANEPPS (Gross et al., 1994; Clarke and Kane 1997; Starke-Peterkovic et al., 2005, 2006; Haldar et al., 2012). Steady state fluorescence measurements were performed with a Hitachi F-7000 (Tokyo, Japan) spectrofluorometer using 1 cm path length quartz cuvettes at room temperature (~23 °C). Excitation and emission slits with a bandpass of 5 nm were used for all measurements. Background intensities of samples were subtracted from each sample to cancel any contribution due to the solvent Raman peak. Fluorescence intensities were recorded at two excitation wavelengths (420 and 520 nm). Emission wavelength was fixed at 670 nm. The fluorescence ratio (R), defined as the ratio of fluorescence intensities at an excitation wavelength of 420 nm to that at 520 nm (emission at 670 nm in both cases) was calculated (Starke-Peterkovic et al., 2006), which is a measure of micellar dipole potential.

2.2.3. Determination of critical micelle concentration

Plots of R *vs.* detergent concentration (or log (detergent concentration) as in Fig. 2a and b) were generated using Origin version 6.0 (OriginLab, Northampton, MA). The variation of R with detergent concentration exhibited sigmoidal dependence (Fig. 2a–c) with initial and final concentrations showing linear dependence. In case of CHAPS (Fig. 2d), the nature of variation of R with detergent concentration was different. CMC was estimated as the

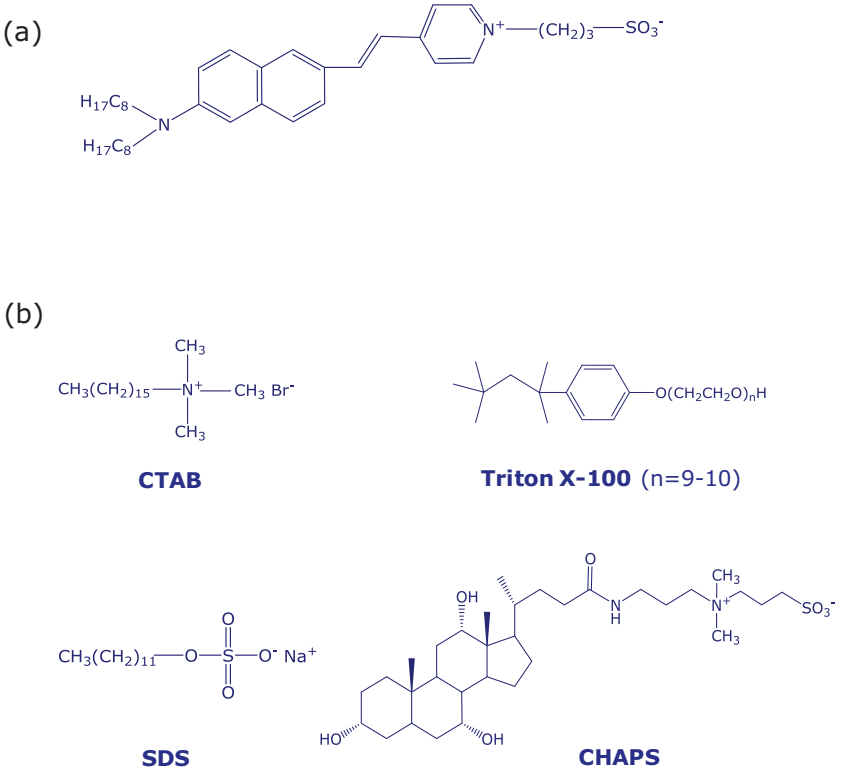

Fig. 1. (a) The structure of the voltage-sensitive probe di-8-ANEPPS. (b) Chemical structures of representative detergents of various charge types used in this study. See text for more details.