Chemistry and Physics of Lipids 191 (2015) 91–95

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Short communication

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

Parijat Sarkar, Amitabha Chattopadhyay*

CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

ARTICLE INFO

Article history:
Received 8 August 2015
Received in revised form 22 August 2015
Accepted 24 August 2015
Available online xxx

Keywords:
CMC
di-8-ANEPPS
Dipole potential
Detergents
Micelles
Dipolar reorganization

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.

* Corresponding author. Tel.: +91 40 2719 2578; fax: +91 40 2716 0311.
E-mail address: amit@ccmb.res.in (A. Chattopadhyay).

http://dx.doi.org/10.1016/j.chemphyslip.2015.08.016
0009-3084/

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

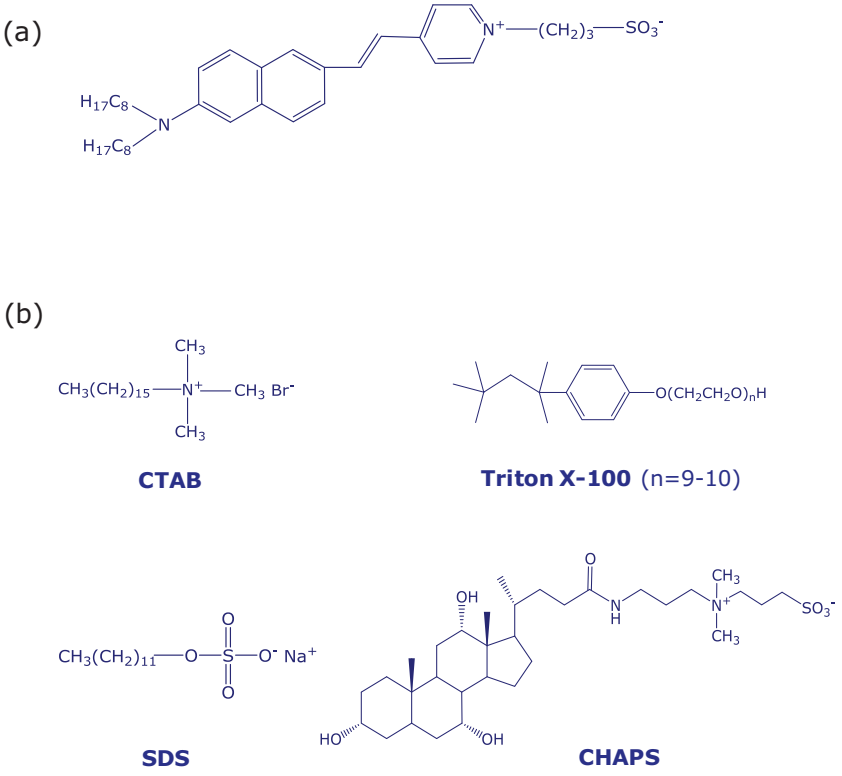

Fig. 1. (a) The structure of the voltage-sensitive probe di-8-ANEPPS. (b) Chemical structures of representative detergents of various charge types used in this study. See text for more details.

corresponding value in the abscissa (x-axis) for the R value at the midpoint ($R_{midpoint}$) between the straight lines obtained at low and high detergent concentrations using the equation:

$$R_{midpoint} = [0.5\ (R_{max} - R_{min})] + R_{min} \quad (1)$$

where R_{max} and R_{min} correspond to the maximum and minimum value of R.

3. Results and discussion

We explored the change in dipole potential associated with the process of micelle formation from detergent monomers. This was carried out by a dual-wavelength ratiometric approach using the voltage-sensitive (electrochromic) styrylpyridinium probe, di-8-ANEPPS (see Fig. 1a) (Gross et al., 1994; Clarke and Kane, 1997; Starke-Peterkovic et al., 2005, 2006). We chose representative detergents of different charge types (see Fig. 1b), *i.e.*, CTAB (cationic), Triton X-100 (neutral), SDS (anionic) and CHAPS (zwitterionic) for these measurements. The detergents chosen display diversity in chemical structure, charge, shape and size of the micelles. Among these, CHAPS is the only synthetic detergent and is a derivative of the naturally occurring bile salts. It combines useful features of both the bile salt hydrophobic group and the *N*-alkyl sulfobetaine type polar group. CHAPS is one of the most commonly used detergents in membrane biochemistry due to its mild, non-denaturing nature (Hjelmeland, 1980; Chattopadhyay et al., 2002).

The dual wavelength ratiometric technique utilizing di-8-ANEPPS is a convenient approach to monitor dipole potential. The fluorescence ratio (R), *i.e.*, the ratio of fluorescence intensities at an excitation wavelength of 420 nm to that at 520 nm, keeping emission constant at 670 nm, has been shown to be sensitive to any change in the dipolar field where the probe is localized. Importantly, since fluorescence intensity is measured using two different excitation wavelengths, R is independent of small changes in dye concentration (Clarke, 2010). R of di-8-ANEPPS is sensitive to any change in the dipolar field due to an electrochromic mechanism, resulting in a shift of di-8-ANEPPS excitation spectrum which could be correlated to the electric field strength (Loew et al., 1979; Le Goff et al., 2007). In addition, it has previously been shown that R of di-8-ANEPPS is sensitive to only dipole potential and is independent of specific molecular interactions (Gross et al., 1994; Robinson et al., 2011).

Fig. 2 shows the change in R of di-8-ANEPPS with increasing detergent concentration for all four detergents chosen for this study. In case of CTAB, Triton X-100 and SDS (Fig. 2a–c), the R value is high at monomeric concentrations of the detergent. The poor signal/noise ratio (as apparent by relatively large error bars), observed in case of low detergent concentrations could be due to relatively low quantum yield of di-8-ANEPPS when incorporated in the pre-micellar state of the detergent. Interestingly, R values exhibit maximum sensitivity to detergent concentration in a narrow range of concentrations around the CMC of the given detergent, indicating profound dipolar reorganization during micelle formation. Upon further increase in detergent concentration (post-micellar concentrations), the R values reach a plateau. To

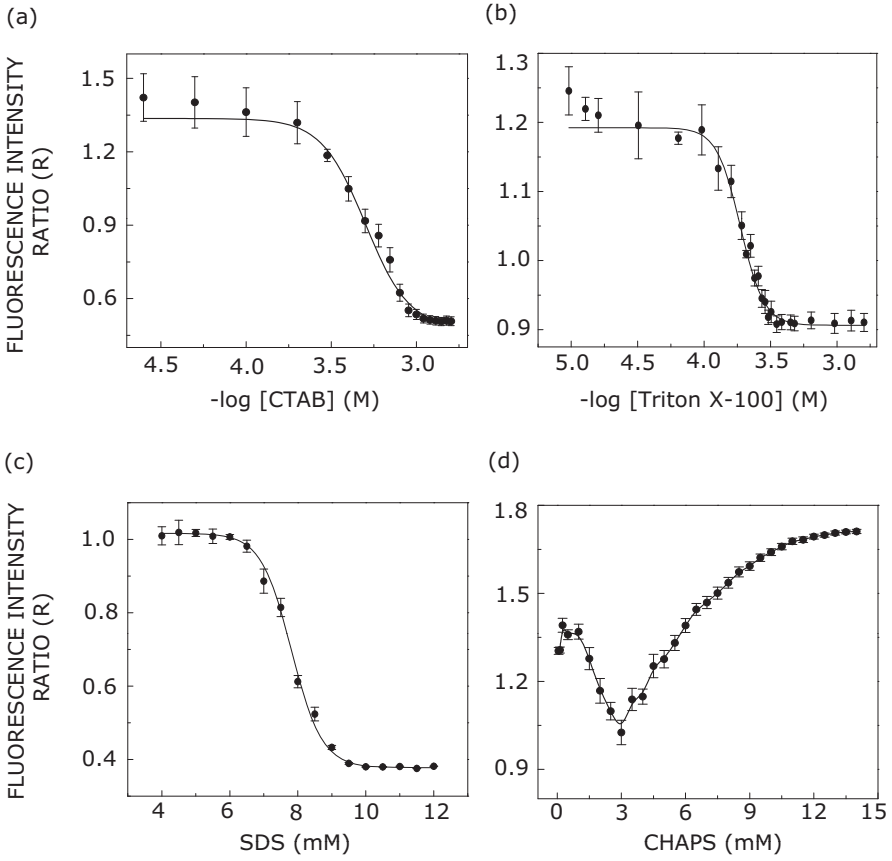

Fig. 2. Change in the fluorescence ratio (R) of the excitation spectra of di-8-ANEPPS with increasing detergent concentration for (a) CTAB, (b) Triton X-100, (c) SDS, and (d) CHAPS. The fluorescence ratio (R) is defined as the ratio of fluorescence intensities at an excitation wavelength of 420 nm to that at 520 nm (emission at 670 nm in both cases; see text). Data points shown are means ± S.E. of at least three independent measurements. The concentration of di-8-ANEPPS was 0.5 μM in all cases. Measurements were carried out at room temperature (~23 °C). The curves in panels (a–c) are nonlinear regression fits to the experimental data, while the lines joining the data points in (d) are provided merely as a viewing guide. See Section 2 for more details.

Table 1
CMC values determined by the ratiometric method and comparison with literature values.

Detergent	Charge	Condition	CMC (present method) (mM)	Literature CMC (mM)
CTAB	Cationic	Water	0.64	0.80[a]
Triton X-100	Neutral	Water	0.21	0.24[b]
SDS	Anionic	Water	7.82	8.00[a]
		10 mM NaCl	3.36	3.50[a]
CHAPS	Zwitterionic	Water	6.25	6.41[c]

[a] Chattopadhyay and London (1984).
[b] Helenius and Simons (1975).
[c] Chattopadhyay and Harikumar (1996).

the best of our knowledge, such drastic changes in dipolar orientation giving rise to characteristic changes in dipole potential (which depends on R) has not been reported previously. In other words, a hallmark of micellization is the dipolar reorganization that results in lower values of R in these cases.

The change in R values upon micellization can be effectively analyzed for determining the CMC of the corresponding detergent (see Section 2.2.3). The CMC values estimated this way are shown in Table 1. The literature values of CMC and the values obtained using this approach are in overall agreement. In order to check whether the CMC values estimated this way are sensitive to ionic strength, we monitored the CMC of the negatively charged detergent SDS using this approach. The CMC of SDS is lowered with increasing salt due to reduced repulsion among its charged headgroups (Chattopadhyay and London, 1984). Table 1 shows that the CMC value of SDS estimated using change in dipole potential with increasing detergent concentration is sensitive to salt, in agreement with previous results.

As mentioned above (see Section 2.2.3), we found the nature of variation of R with detergent concentration was different in case of the zwitterionic detergent CHAPS (see Fig. 2d). The R value exhibited a reduction with detergent concentration up to a certain concentration (~3 mM) of CHAPS. Increase in detergent concentration beyond 3 mM resulted in a concentration-dependent increase in R value which gradually leveled off at high detergent concentrations. We attribute this type of dual mode of change in R with CHAPS concentration to the reported ability of bile acid derivatives such as CHAPS to form two types of micellar organization, depending on detergent concentration. At low concentration (up to ~3 mM), CHAPS forms small lateral aggregates from dimers to octamers (termed 'primary micelles') in which the detergent monomers lie back to back (Carey and Small, 1972; Helenius and Simons, 1975; Barnadas-Rodríguez and Cladera, 2015). At higher CHAPS concentrations, CHAPS forms 'secondary micelles' which are larger aggregates formed from the initial lateral aggregates (primary micelles) held together by polar interactions. Such a dual concentration-dependent micellar organization of CHAPS has recently been reported using nuclear magnetic resonance spectroscopy (Funasaki et al., 2006), spin label electron paramagnetic resonance spectroscopy (Rodi et al., 2014) and molecular dynamics simulation (Herrera et al., 2014). We therefore estimated two CMC values for CHAPS, the first one at low detergent concentration (CMC ~1.77 mM) corresponding to small lateral aggregates and the second CMC (~6.25 mM) at higher concentrations. Table 1 shows the CMC value for CHAPS estimated this way for secondary micelles, in excellent agreement with the literature value of 6.41 mM (Chattopadhyay and Harikumar, 1996). It is interesting to note here that the present method is capable of distinguishing between these two types of micelle formation (*i.e.*, primary and secondary micelles) based on the sensitivity of dipole potential to dipolar reorganizations associated with the formation of these two types of micelles (evident from the opposite trends in change in R with detergent concentration, see Fig. 2d). The differential patterns of change in dipole potential, depending on detergent concentration, in case of CHAPS could be indicative of the different modes of association of CHAPS monomers in forming the primary and secondary micelles.

In summary, we report here that the process of micelle formation involves dipolar reorganization which could be monitored using change in ratiometric fluorescence of the potential-sensitive membrane probe di-8-ANEPPS. In addition, such changes in dipolar reorganization, monitored using ratiometric fluorescence can be further analyzed to obtain CMC of a variety of detergents, displaying diversity in chemical structure, shape, charge, and micellar size. We show here that the change in ratiometric fluorescence of the potential-sensitive dye di-8-ANEPPS, which provides a handle for dipole potential, could be a sensitive indicator of micelle formation. Although the concept of dipole potential has been previously applied to biological and model membranes (Gross et al., 1994; Starke-Peterkovic et al., 2006; Haldar et al., 2012; Richens et al., 2015), the use of dipole potential to explore micellar properties represents a new and exciting possibility. We envision that application of dipole potential could open novel ways to explore micellar assemblies.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by the Council of Scientific and Industrial Research, Govt. of India. P.S. thanks the Council of Scientific and Industrial Research for the award of a Shyama Prasad Mukherjee Fellowship. A.C. is an Adjunct Professor of Tata Institute of Fundamental Research (Mumbai), RMIT university (Melbourne, Australia), Indian Institute of Technology (Kanpur), and Indian Institute of Science Education and Research (Mohali). A.C. gratefully acknowledges J.C. Bose Fellowship (Department of Science and Technology, Govt. of India). We thank members of the Chattopadhyay laboratory for their comments and discussions.

References

Bandari, S., Chakraborty, H., Covey, D.F., Chattopadhyay, A., 2014. Membrane dipole potential is sensitive to cholesterol stereospecificity: implications for receptor function. Chem. Phys. Lipids 184, 25–29.
Barnadas-Rodríguez, R., Cladera, J., 2015. Steroidal surfactants: detection of premicellar aggregation, secondary aggregation changes in micelles, and hosting of a highly charged negative substance. Langmuir 31, 8980–8988.
Brockman, H., 1994. Dipole potential of lipid membranes. Chem. Phys. Lipids 73, 57–79.
Carey, M.C., Small, D.M., 1972. Micelle formation by bile salts: physical–chemical and thermodynamic considerations. Arch. Intern. Med. 130, 506–527.
Chattopadhyay, A., London, E., 1984. Fluorimetric determination of critical micelle concentration avoiding interference from detergent charge. Anal. Biochem. 139, 408–412.

Chattopadhyay, A., Harikumar, K.G., 1996. Dependence of critical micelle concentration of a zwitterionic detergent on ionic strength: implications in receptor solubilization. FEBS Lett. 391, 199–202.
Chattopadhyay, A., Harikumar, K.G., Kalipatnapu, S., 2002. Solubilization of high affinity G-protein-coupled serotonin$_{1A}$ receptors from bovine hippocampus using pre-micellar CHAPS at low concentration. Mol. Membr. Biol. 19, 211–220.
Chattopadhyay, A., Rao, B.D., Jafurulla, M., 2015. Solubilization of G protein-coupled receptors: a convenient strategy to explore lipid–receptor interaction. Methods Enzymol. 557, 117–134.
Chaudhuri, A., Chattopadhyay, A., 2014. Lipid binding specificity of bovine α-lactalbumin: a multidimensional approach. Biochim. Biophys. Acta 1838, 2078–2086.
Chaudhuri, A., Haldar, S., Chattopadhyay, A., 2009. Organization and dynamics in micellar structural transition monitored by pyrene fluorescence. Biochem. Biophys. Res. Commun. 390, 728–732.
Cladera, J., O'Shea, P., 1998. Intramembrane molecular dipoles affect the membrane insertion and folding of a model amphiphilic peptide. Biophys. J. 74, 2434–2442.
Clarke, R.J., 2001. The dipole potential of phospholipid membranes and methods for its detection. Adv. Colloid Interface Sci. 89-90, 263–281.
Clarke, R.J., 2010. Electric field sensitive dyes. In: Demchenko, A.P. (Ed.), Advanced Fluorescence Reporters in Chemistry and Biology I: Fundamentals and Molecular Design. Springer, Heidelberg, pp. 331–344.
Clarke, R.J., Kane, D.J., 1997. Optical detection of membrane dipole potential: avoidance of fluidity and dye-induced effects. Biochim. Biophys. Acta 1323, 223–239.
Duffin, R.L., Garrett, M.P., Flake, K.B., Durrant, J.D., Busath, D.D., 2003. Modulation of lipid bilayer interfacial dipole potential by phloretin, RH421, and 6-ketocholestanol as probed by gramicidin channel conductance. Langmuir 19, 1439–1442.
Funasaki, N., Fukuba, M., Hattori, T., Ishikawa, S., Okuno, T., Hirota, S., 2006. Micelle formation of bile salts and zwitterionic derivative as studied by two-dimensional NMR spectroscopy. Chem. Phys. Lipids 142, 43–57.
Gross, E., Bedlack Jr, R.S., Loew, L.M., 1994. Dual-wavelength ratiometric fluorescence measurement of the membrane dipole potential. Biophys. J. 67, 208–216.
Haldar, S., Kanaparthi, R.K., Samanta, A., Chattopadhyay, A., 2012. Differential effect of cholesterol and its biosynthetic precursors on membrane dipole potential. Biophys. J. 102, 1561–1569.
Helenius, A., Simons, K., 1975. Solubilization of membranes by detergents. Biochim. Biophys. Acta 415, 29–79.
Herrera, F.E., Garay, A.S., Rodrigues, D.E., 2014. Structural properties of CHAPS micelles, studied by molecular dynamics simulations. J. Phys. Chem. B 118, 3912–3921.
Hjelmeland, L.M., 1980. A nondenaturing zwitterionic detergent for membrane biochemistry: design and synthesis. Proc. Natl. Acad. Sci. U. S. A. 77, 6368–6370.
Kalipatnapu, S., Chattopadhyay, A., 2005. Membrane protein solubilization: recent advances and challenges in solubilization of serotonin$_{1A}$ receptors. IUBMB Life 57, 505–512.
Le Goff, G., Vitha, M.F., Clarke, R.J., 2007. Orientational polarisability of lipid membrane surfaces. Biochim. Biophys. Acta 1768, 562–570.
Loew, L.M., Scully, S., Simpson, L., Waggoner, A.S., 1979. Evidence for a charge-shift electrochromic mechanism in a probe of membrane potential. Nature 281, 497–499.
Neugebauer, J.M., 1990. Detergents: an overview. Methods Enzymol. 182, 239–253.
O'Shea, P., 2005. Physical landscapes in biological membranes: physico-chemical terrains for spatio-temporal control of biomolecular interactions and behavior. Philos. Trans. R. Soc. Lond. A 363, 575–588.
Raghuraman, H., Chattopadhyay, A., 2004. Effect of micellar charge on the conformation and dynamics of melittin. Eur. Biophys. J. 33, 611–622.
Rawat, S.S., Kelkar, D.A., Chattopadhyay, A., 2005. Effect of structural transition of the host assembly on dynamics of an ion channel peptide: a fluorescence approach. Biophys. J. 89, 3049–3058.
Richens, J.L., Lane, J.S., Bramble, J.P., O'Shea, P., 2015. The electrical interplay between proteins and lipids in membranes. Biochim. Biophys. Acta 1848, 1828–1836.
Rivnay, B., Metzger, H., 1982. Reconstitution of the receptor for immunoglobulin E into liposomes. Conditions for incorporation of the receptor into vesicles. J. Biol. Chem. 257, 12800–12808.
Robinson, D., Besley, N.A., O'Shea, P., Hirst, J.D., 2011. Di-8-ANEPPS emission spectra in phospholipid/cholesterol membranes: a theoretical study. J. Phys. Chem. B 115, 4160–4167.
Rodi, P.M., Bocco Gianello, M.D., Gennaro, A.M., 2014. Insights about CHAPS aggregation obtained by spin label EPR spectroscopy. Appl. Magn. Reson. 45, 1319–1332.
Sham, S.S., Shobana, S., Townsley, L.E., Jordan, J.B., Fernandez, J.Q., Andersen, O.S., Greathouse, D.V., Hinton, J.F., 2003. The structure, cation binding, transport and conductance of Gly$_{15}$-gramicidin A incorporated into SDS micelles and PC/PG vesicles. Biochemistry 42, 1401–1409.
Singh, P., Haldar, S., Chattopadhyay, A., 2013. Differential effect of sterols on dipole potential in hippocampal membranes: implications for receptor function. Biochim. Biophys. Acta 1828, 917–923.
Starke-Peterkovic, T., Clarke, R.J., 2009. Effect of headgroup on the dipole potential of phospholipid vesicles. Eur. Biophys. J. 39, 103–110.
Starke-Peterkovic, T., Turner, N., Else, P.L., Clarke, R.J., 2005. Electric field strength of membrane lipids from vertebrate species: membrane lipid composition and Na^+–K^+-ATPase molecular activity. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288, R663–R670.
Starke-Peterkovic, T., Turner, N., Vitha, M.F., Waller, M.P., Hibbs, D.E., Clarke, R.J., 2006. Cholesterol effect on the dipole potential of lipid membranes. Biophys. J. 90, 4060–4070.
Tanford, C., 1978. The hydrophobic effect and the organization of living matter. Science 200, 1012–1018.
Wang, L., 2012. Measurements and implications of the membrane dipole potential. Annu. Rev. Biochem. 81, 615–635.