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Research Paper

Micellar dipolar rearrangement is sensitive to hydrophobic chain length: Implication for structural switchover of piroxicam

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ABSTRACT

The interfacial properties of the membrane are exceptionally vital in drug-membrane interaction. They not only select out a particular prototropic form of the drug molecule for incorporation, but are also potent enough to induce structural switchover of these drugs in several cases. In this work, we quantitatively monitored the change in dipolar rearrangement of the micellar interface (as a simplified membrane mimic) by measuring the dielectric constant and dipole potential with the micellization of SDS at pH 3.6. The dielectric constant and dipole potential were measured utilizing the fluorescence of polarity sensitive probe, pyrene and potential-sensitive probe, di-8-ANEPPS, respectively. Our study demonstrates that the change in dipolar rearrangement directly influences the switchover equilibrium between the anionic and neutral from of piroxicam. We have further extended our work to evaluate the effect of hydrophobic chain length of the surfactants on the dipolar rearrangement and its effect on the structural switchover of piroxicam. It is interesting that the extent of switchover of piroxicam is directly correlated with the dipolar rearrangement induced bythe varying hydrophobic chain length of the surfactants. To the best of our knowledge, our results constitute the first report to show the dependence of dipole potential on the hydrophobic chain length of the surfactant and demonstrate that the dipolar rearrangement directly tunes the extent of structural switchover of piroxicam, which was so far only intuitive. We consider that this new finding would have promising implication in drug distribution and drug efficacy.

1. Introduction

The exciting chemistry of the membrane interface is instrumental for different biological functions of the membrane, as membranes are considered to be the unique meeting place of lipids and proteins (Escriba et al., 2008). The unique environment of the interface is formed by the diverse headgroup chemistry of the lipid molecules and the intermolecular hydrogen bonding between water molecules and lipid headgroup. A typical environment of the membrane interface is characterized by its polarity, anisotropy, dielectric constant, surface potential, hydrogen bonding ability, heterogeneity and several other physical parameters (Chattopadhyay, 2003; Falck et al., 2004; Mukherjee et al., 2007; Mukherjee and Maxfield, 2004). It has been recently shown that dipole potential is a critical parameter that maneuvers the chemistry at the interface. Dipole potential corresponds to the potential difference within the membrane bilayer. The non-random orientation of electric dipoles of lipid and water molecules is the origin of membrane dipole potential (Brockman, 1994; Clarke, 2001; O'Shea, 2005; Wang, 2012). Dipole potential operates over short range and varies between 200 and 1000 mV, depending on membrane composition. Membrane dipole potential has been reported to be a sensitive indicator of the function of membrane proteins and peptides and is often used to monitor the binding of proteins to membranes (Chaudhuri and Chattopadhyay, 2014; Cladera and O'Shea, 1998).

The oxicam group of Non Steroidal Anti-Inflammatory Drugs (NSAIDs) were initially used against pain and inflammation but over the last decade these drugs have drawn colossal attention because of their multifunctional efficiencies (Chakraborty et al., 2007, 2008; Grossman et al., 2000; Hoozemans et al., 2002; Ritland and Gendler, 1999; Roy et al., 2006; Sagi et al., 2003; Sporn and Suh, 2000; Weggen et al., 2001). These functions include chemoprevention and chemosuppression in several cancer cell lines as well as in animal models (Grossman et al., 2000; Ritland and Gendler, 1999; Sporn and Suh, 2000) and protective effect against

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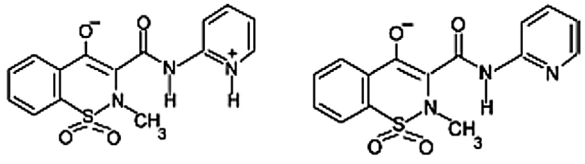
neurodegenerative diseases (Hoozemans et al., 2002; Sagi et al., 2003; Weggen et al., 2001). The versatility of these drugs are often attributed to the existence of their different prototropic forms and the microenvironment sensitivity of each of these forms (Chakraborty et al., 2003, 2007, 2005). The oxicam group of NSAIDs show microenvironment-dependent switchover between different prototropic forms without changing the pH of the bulk solution (Chakraborty et al., 2003; Chakraborty and Sarkar, 2004). The extent of switchover depends on the micellar charge (Chakraborty et al., 2003; Chakraborty and Sarkar, 2004), salt concentration (Chakraborty and Sarkar, 2005a) and hydrophobic chain length (Chakraborty and Sarkar, 2005b). The principal targets of NSAIDs are cyclooxygenases (COXs) enzymes, which are a class of membrane associated proteins (Hawkey, 1999). Therefore, these molecules, at first, interact with the membrane, and the properties of the membrane decide a particular prototropic form to be presented to the target. In our present work, we have chosen piroxicam as a representative molecule of oxicam group of NSAIDs. Piroxicam (see Scheme 1) demonstrates the structural and functional multiplicities like other oxicam group of NSAIDs, and the predominance of a particular prototropic form is extremely sensitive to the microenvironment (Chakraborty et al., 2003). In addition, interaction of piroxicam with mitochondrial membranes leads to mitochondrial fusion followed by rupture of the mitochondrial membrane and induction of apoptosis (Chakraborty et al., 2007).

The importance of microenvironment is enormous for the activity of the drug molecules. The interfacial properties of the membrane act as a molecular switch for the oxicam group of NSAIDs and a particular prototropic form of piroxicam is chosen over the other depending on the membrane microenvironment (Chakraborty et al., 2003, 2008; Chakraborty and Sarkar, 2004). These molecules have also drawn massive attention in drug industry because of their multifunctional character. Modulation of interfacial properties by changing the polar head-group and solvent properties is very common, but there is not enough evidence in support of alteration of interfacial properties of the membrane by altering the hydrophobic chain length. In addition, though there are several evidences of microenvironment-dependent switchover but the extent of switchover equilibrium was hardly correlated with any interfacial physical parameter. In this paper, we have measured the dipolar rearrangement in terms of dielectric constant and dipole potential of sodium dodecyl sulphate (SDS) micelles during the process of micellization and correlated these parameters with the extent of structural switchover of piroxicam. We have further extended these measurements to a series of surfactants (SnS) with differing hydrophobic chain length and correlated the dipolar rearrangement in micelles with the extent of switchover of piroxicam in those micelles. Altogether, this work helps us to understand and correlate two important concepts of surface chemistry that are extremely important in drug-membrane interactions viz., (i) dependence of the dipolar rearrangements of the micelles, especially dipole potential on the hydrophobic chain length of the surfactant and (ii) application of dipolar rearrangement as a molecular switch for the structural switchover ofpiroxicam, which was completely intuitive in the earlier results.



Scheme 1. Structure of Zwitterionic and Anionic forms of Piroxicam.

2. Materials and method

S12S, S10S, S8S, and pyrene were obtained from Sigma Chemical Co. (St. Louis, MO). 4-(2-(6-(Dioctylamino)-2-naphthalenyl) ethenyl)- 1-(3-sulfopropyl)-pyridinium inner salt (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.

Absorption spectra were recorded with a Shimadzu UV–vis spectrophotometer model UV2410. Baseline correction wasperformed with water before measuring each set of data. Fluorescence measurements were performed using a Hitachi spectrofluorometer model F-7000 (Tokyo, Japan). All emission spectra were corrected for instrument response at each wavelength. The concentration of the drug was measured from Lambert-Beer's law, as the extinction coefficients are known at the characteristic wavelength of the global neutral and anionic forms (Banerjee et al., 2003). A $2 \times 10\,mm^2$ path length quartz cell was used for all fluorescence measurements to avoid any blue edge distortion of the spectrum due to inner filter effect (Lakowicz, 1999).

2.1. Sample preparation

Surfactant solutions were prepared in a total volume of 2 ml 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 surfactants) and mixed well by vortexing for 1 min. The resultant di-8-ANEPPS concentration was 2 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. All measurements were carried out at pH 3.6, where piroxciam exhibits microenvironment dependent structural switchover (Chakraborty et al., 2003). Experiments were repeated with atleast three sets of samples at room temperature (~23 °C).

2.2. Measurement of environment polarity from pyrene fluorescence intensity ratio

Steady state fluorescence measurements of samples containing pyrene were performed with a Hitachi F-7000 spectrofluorometer using 1 cm path length quartz cuvettes. For measuring pyrene fluorescence, samples were excited at 335 nm. Excitation and emission slits with a nominal bandpass of 3 nm were used for all measurements. The background intensities of samples in which pyrene was omitted were measured and subtracted from each sample spectrum to cancel out any contribution due to the solvent Raman peak and other scattering artefacts. Due to the small size of micelles, samples were relatively scatter-free. The ratio of the first (373 nm) and third (384 nm) vibronic peak intensities (I_1/I_3) was calculated from pyrene emission spectra. The fluorescence intensity ratio (I_1/I_3) is extremely sensitive to the environmental polarity and can directly be correlated with the dielectric constant of the medium (Dong and Winnik, 1982).

2.3. 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 (Clarke and Kane, 1997; Gross et al., 1994; Haldar et al.,