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Molecular dynamics of the interaction of anionic surfactants with liposomes

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ABSTRACT

The time dependent molecular interactions of sodium dodecyl sulfate (SDS) with dipalmitoylphosphatidylcholine (DPPC) liposomes were studied using attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy. In particular, the ATR spectra provided information on the dynamic amount of SDS incorporated and DPPC expelled in the DPPC structure along with structural information on the molecular nature of the SDS/DPPC assemblies. It was found that the uptake of SDS was always mirrored by the ejection of DPPC molecules from the liposomes and that the incorporation of SDS followed two routes; SDS intercalated into the DPPC liposomes and SDS was bound electrostatically to the charged surface of the DPPC liposome.

1. Introduction

Surfactants find use as solubilizing agents in the isolation, purification, reconstitution and crystallization of membrane proteins [1–5]. They also have been incorporated in the membrane of drug-carrying liposomes to alter its permeability and thus affecting their behavior with respect to drug release [6–10]. As a result, the interaction of surfactants with model phospholipid liposomes have been the subject of several studies [11–30] aimed at gaining a molecular understanding of the mechanism of surfactant induced membrane dissolution.

It is well known that the exposure of liposomes to surfactants leads to their rupture resulting in the solubilization of the phospholipids. The generally accepted process for the dissolution of the liposomes follows three stages [12–15,31]. In stage I, the surfactants adsorb on the liposomes and intercalate into the lipid bilayer. The second stage occurs when the surfactant level incorporated in the bilayer exceeds a critical value which initiates the formation and detachment of phospholipid–surfactant mixed micelles. The void produced by the expulsion of the mixed micelle is filled by surfactants and lipids transferring from the inner to the outer membrane layer. Stage III occurs at the completion of the liposome-to-micelle transition in which the dissolution of the vesicle occurs. In this final stage, all phospholipids are present as mixed micelles.

Much of the understanding of surfactant–membrane interactions is derived from measurements of the dissolution of vesicles as a function of surfactant concentration and contact time [12–29,32,33]. A model system often studied is the interaction of the anionic surfactant, sodium dodecyl sulfate (SDS) with simplified membrane models of phosphatidylcholine (PC) liposomes. Techniques such as dynamic light scattering [12,17,22,27,28,30], electron paramagnetic resonance (EPR) [25,32], fluorescence spectroscopy [12,17–19] and small-angle X-ray scattering [11,23] have been used to follow changes in the permeability and size of the liposomes when exposed to solutions containing SDS.

Deo and coworkers [14] measured changes in optical density of liposome suspensions and the concentration of free SDS monomers in the SDS–PC liposome system. From this work, they concluded that liposome solubilization by SDS occurs by adsorption of SDS onto the liposome external surface followed by intercalation of SDS into the membrane which, in turn, initiates dissolution of the liposome to form mixed micelles. The same conclusions were reached by Cocera and coworkers [18] using data obtained from dynamic light scattering and fluorescence spectroscopy. These authors also found that adsorption of SDS by the liposomes was rapid in agreement with other studies [12,19,24,27].

Recently, we have used ATR-FTIR to study dynamically the interaction of surfactants and polymers on TiO_2 particulates [34–37]. While the dynamic change in the amount of surfactant and polymer adsorbed on TiO_2 was spectroscopically measured, it was when this data was coupled with the structural information obtained

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from the change occurring in IR bands due to the headgroup of surfactants, that a much clearer molecular description emerged. This same approach has been used to study CTAB adsorption on TiO_2 [35], mixed CTAB/SDS on TiO_2 [36], and polymer/surfactant interactions [34,37].

We have also used this ATR-FTIR approach to study the nature of the interaction of DPPC liposomes deposited on TiO_2 [38]. As found with the surfactant/polymer studies, the nature of the DPPC structure was determined from analysis of the headgroup IR bands of DPPC. This study showed that the liposomes remain intact on the TiO_2 and are not removed by flowing water solutions. This finding was confirmed in another ATR-FTIR study that used liposomes containing probe molecules trapped in the interior cavity [39].

A natural extension of these ATR-FTIR studies is to investigate the dynamic interaction of liposomes with various molecules. In our first ATR-FTIR study we examined the interaction of cholesterol, ergosterol and 7-dehydrocholesterol with EggPC and DPPC liposomes [40]. In this study, we now use the ATR-FTIR based technique is used to provide molecular evidence of the perturbation of DPPC liposomes to solutions containing SDS. Specifically, the amount of SDS incorporated in the DPPC structure and the amount of DPPC expelled is measured as a function of contact time. At the same time, analysis of changes in the headgroup IR bands of both SDS and DPPC provides structural information on the nature of the SDS/DPPC assemblies. We show that SDS both adsorbs on the outer liposome surface and intercalates into the DPPC layer. The final structure that was generated by the interaction of SDS with the liposomes depends on the solution concentration of SDS. Although there are several IR studies of phospholipids [41–48], to the best of our knowledge, this is the first time it has been used to measure dynamically the structure and conformation changes of liposome/SDS systems.

2. Materials and methods

2.1. Materials

Dipalmitoylphosphatidylcholine (DPPC), obtained from Avanti Polar Lipids, Inc. (Alabaster, AL) with a purity of 99%, was used without further purification. Sodium dodecyl sulfate (SDS) was obtained from Sigma–Aldrich and recrystallized twice from acetone prior to use. Fumed titanium dioxide (P25, surface area of $50\,m^2/g$) was obtained from Degussa and had a measured isoelectric point (IEP) of pH 6.8. Spectrophotometric grade $CHCl_3$ and CH_3OH were purchased from Fisher Scientific. Deionized water (18 MΩ) was obtained from a Milli-Q purification system. Tris HCl buffer solution (pH 8.5) was purchased from K.D Medical Inc., Columbia, MD and used by diluting to 50 mM.

2.2. Substrate pretreatment

The ZnSe internal reflection element (IRE) was obtained from Harrick Scientific, and has dimensions of 50 mm × 10 mm × 2 mm, with 45° beveled faces. A TiO_2 layer (approx. 500 nm thickness) on ZnSe IRE was prepared using a previous method [38] and it is stable, showing no loss of powder to flowing aqueous solutions containing polyelectrolytes and surfactants over a pH range of 2–10 [49].

2.3. Liposome preparation

Unilamellar liposomes of uniform size were prepared by the method described elsewhere [50]. In brief, a lipidic film was formed by rotary evaporation of a lipid/$CHCl_3$ (200 mg in 6 ml) solution. The film was then hydrated at 50 °C overnight in 20 ml deionized water followed by a 10-fold passage through 200 nm polycarbonate membranes. After preparation, the size distribution curves of liposomes (measured on a Malvern Zetasizer 3000 system) varied little (monomodal distribution with a hydrodynamic diameter of about 190 nm, see Fig. S1 in supporting information), the polydispersity index (PI) always showing values lower than 0.1. This indicated a homogeneous particle size distribution.

2.4. ATR-FTIR study

ATR experiments were conducted using a standard ATR flow-through accessory obtained from Harrick mounted into a Bomem MB-series FTIR spectrometer equipped with a liquid N_2-cooled mercury cadmium telluride (MCT) detector. The TiO_2 coated ZnSe IRE was assembled into the standard ATR liquid flow cell. All IR spectra were recorded at a resolution of $4\,cm^{-1}$ with 100 coadded scans.

In a typical experiment, a water solution at pH 8.5 was flowed at a rate of 3 ml/min through the ATR setup for at least 2 h. The reason for operating at pH 8.5 is provided in the supporting information. A reference spectrum was then recorded with water flowing through the ATR cell. Next, a DPPC liposome solution at pH 8.5 was then flowed through the cell and spectra were recorded as a function of time. After 5 h incubation in a flowing DPPC liposome solution, there were no further changes in the bands due to the DPPC observed with longer incubation times. After achieving a maximum in the amount of DPPC liposomes adsorbed on TiO_2, the cell was then flushed with water for 10 min, followed by addition of a fresh solution of SDS at the specified concentration, pH 8.5 and flow rate of 3 ml/min. Spectra were recorded as a function of contact time with SDS for a total period of 6–10 h.

Experiments were conducted at three different SDS solution concentrations, 0.6 mM, 8 mM and 50 mM. The 0.6 mM concentration is well below the critical micelle concentration (CMC) of 7 mM SDS in pure water and it has been shown that the uptake of SDS at this solution concentration range does not lead to dissolution of the DPPC liposome [12,14,24]. The 8 mM concentration was selected because it is just above the CMC whereas the 50 mM is well above the CMC and uptake of SDS at 50 mM leads to dissolution of DPPC liposomes [12,14,24]. All measurements were performed at 24 ± 1 °C and each experiment was repeated a minimum of three times.

The amount of SDS and DPPC adsorbed was determined using the integrated intensity of the CH_2 band at $2850\,cm^{-1}$. In this study, the amount of DPPC was presented as the number of mmoles of DPPC per gram of TiO_2 while the amount of SDS was presented as the number of SDS molecules per number of DPPC molecules in the outer layer of the liposome for developing a molecular description of SDS interactions with the DPPC liposomes. Representative spectra along with the details in the calculation of the adsorbed amount and computing the number of DPPC molecules in the outer layer are discussed in the supporting information. Spectra were recorded using SDS solutions at various concentrations through the ATR setup containing a bare TiO_2 coated ZnSe IRE at pH 8.5. The data obtained (see Fig. S3) is provided in the supporting information and show that SDS does not adsorb on the TiO_2 at pH 8.5. At 0.6 mM SDS bands due to SDS were not detected. However, at 8 and 50 mM SDS concentrations, bands due to SDS were observed but are due to SDS in the solution phase. The spectrum recorded for SDS in the solution phase was then subtracted from the overall spectrum recorded during subsequent experiments involving adsorbed liposomes. It is noted that this correction was small, accounting for less than 15% of the total band intensity at the highest solution concentration of 50 mM SDS.