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Biophysical investigation of the interfacial properties of cationic fluorocarbon/hydrocarbon hybrid surfactant: Mimicking the lung surfactant protein C

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ABSTRACT

The interfacial behavior of the newly designed Fluorocarbon Hydrocarbon Cationic Lipid (FHCL or $CH_3(CH_2)_{17}N^+(C_2H_5)_2(CH_2)_3(CF_2)_7CF_3I^-$) and its mixtures with a phospholipid (DPPC, Dipalmitoylphosphatidylcholine) at different mole fractions were investigated. This new molecule was synthesized to mimic the selected properties of lung surfactant, which is a natural lipid–protein mixture which is known to play important roles in the process of respiration, by considering the structure/function relation of lung surfactant protein (SP-C). Each segment in the molecular structure was selected to affect the molecular level interaction at the interface whereas the keeping the overall structure as simple as possible. The surface pressure area isotherms obtained for the mixtures of DPPC/FHCL indicated that there was repulsive interaction between DPPC and FHCL molecules. Due to the molecular level interaction, specifically at mole fraction 0.3, the isotherm obtained from that mixture resembled the isotherm obtained from the DPPC monolayer in the presence of SP-C. High elasticity of the interface was one of the important parameters for the respiration process, therefore, shear and dilatational elasticities of two-component systems were determined and they were found to be similar to the case where SP-C protein is present. Fluorescence microscopy images were taken in order to investigate the monolayer in details. The FHCL was able to fluidize the DPPC monolayer even at high surface pressures effectively. In addition, the cyclic compression–expansion isotherms were obtained to understand the spreading and re-spreading ability of the pure FHCL and the mixed DPPC/FHCL monolayers. At a specific mole fraction, $X_{FHCL} = 0.3$, the mixture exhibited good hysteresis in area, compressibility, recruitment index and re-spreading ability at the interface. All these results point out that FHCL can fulfill the selected features of the lung surfactant that are attributed to the presence of SP-C protein when mixed with DPPC, even if the molecular structure of the FHCL is quite simple.

1. Introduction

Surface Active Agents are common materials used in chemical and pharmaceutical industry for different purposes as well as in several end products that we use in our daily life. Moreover, there are several examples in nature where surfactants play a crucial role. Lung (or Pulmonary) surfactant is a good example to natural surfactants whose presence is vital for a healthy life. Lung surfactant is a mixture of lipids and proteins that cover the alveolar surface of lungs lower surface tension and prevent alveolar collapse during respiration [1–5]. This complex mixture contains ~85% phospholipids, ~5% neutral lipids and ~10% proteins (SP-A, SP-B, SP-C and SP-D) [2,5]. Each component in the mixture has different function during respiration process. Lipids can form a condensed monolayer by adsorbing to the air–water interface of alveoli and reduce surface tension. Although these lipids in the mixture, especially the most abundant one – DPPC (Dipalmitoylphosphatidylcholine) – have unique properties; they are not sufficient by themselves to maintain a stable respiration cycle. DPPC is sensible to mechanical disturbance and collapses irreversibly when over compressed. In addition, it adsorbs very slowly from the aqueous suspension and does not re-spread when compression is relieved due to strong intermolecular interactions. Thus, assistance of pulmonary proteins (two hydrophobic and two hydrophilic proteins) is vital to provide a sustainable respiration cycle. Hydrophilic proteins (SP-A, SP-D) take role in immune system and transportation of pulmonary surfactant mixture from pulmonary cell to the interface. Hydrophobic proteins (SP-B, SP-C) are responsible for monolayer properties which are imperative for healthy breathing [2,5]. SP-C, a protein with a cationic character having two palmitoyl chains and α-helix structure, has a specific transbilayer orientation and helps to regulate the elasticity and fluidity of the monolayer [3,4]. The compression of the alveolar surface leads to the exclusion of some material from the interface, resulting in the formation of bilayer and multilayered structures of surfactants that remain

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associated with the interface. This multilayer formation is significantly enhanced by the presence of the hydrophobic SP-C protein. This multilayer act as a surfactant reservoir and during expansion cycle allows the surfactant molecules to rapidly come to the interface [6,7]. Because of the difficulties on the synthesis of this protein several analogs of it have been synthesized and reported in literature [8,9]. SP-B, also has a cationic character, helps to transport DPPC molecules to the interface from sub-phase and holds the collapsed DPPC molecules at sub-interface. Thus, DPPC molecules can easily re-spread into the interface during expansion [1,2,10].

The deficiency or change in the composition of the pulmonary surfactant results in several fatal conditions such as neonatal respiratory distress syndrome (NRDS) or acute respiratory distress syndrome (ARDS) [5,11]. Exogenous surfactant therapies are known to reduce the mortality rates significantly in RDS. However, exogenous treatment is not in use for ARDS yet. The pulmonary surfactant can be extracted from animals but animal derived mixture could have several disadvantages such as the risk of infection, transmission of diseases, variations of the content from batch to batch. Moreover, the extraction process could be costly. For all that, there are several synthetic mixtures which contain DPPC and several co-additives to adjust the characteristics of the mixture and try to fulfill the behavior of the real system [12–14]. In some studies which are focused on ARDS, to restore pulmonary functions that have been inactivated by serum proteins or other environmental conditions, utilization of polymers or long chain alcohols to adjust the colloidal stability and adsorption of lung surfactant have been investigated [15–19]. It is possible to find some studies in literature that are focused on the basic function/malfunction of lung surfactant and specifically the effect of the SP-B protein [20]. However, a study on a mixture, where the structure of the molecules is simple but still posses enough ability to interact with one another, which can fulfill the properties of the lung surfactant associated with the presence with the SP-C protein is still needed. For example, DPPC can be used as a lung expansion agent but will not be useful to capture all the necessary function. This is the driving force of the most study conducted nowadays to capture the selected properties of the real mixture by utilizing synthetic formulations [14,21]. In the study presented here it is aimed to create an understanding on the selected functions of lung surfactant that are associated with the SP-C protein and making simple connection of these with molecular level interaction of lipids with the designed surfactant molecule. By doing that it is not only aimed to mimic a real system but also creating a basic understanding on the behavior of lung surfactant associated with the presence of SP-C protein.

Fluorocarbons show exciting behavior when they are added to the molecular structure or used by themselves. They are very stable at severe environmental conditions, biologically inert and able to solubilize oxygen. Fluorocarbons are more hydrophobic than hydrocarbons. This increased hydrophobicity is primarily a matter of low polarizability of the fluorocarbon chains. Moreover, they are lipophobic [22,23]. They can fluidize the lipid monolayer and this property of fluorocarbons can be used to capture the fluid like behavior of lung surfactants [24,25]. Fluorinated surfactants have the tendency to reduce the surface tension to very low value. They tend to self-assemble more easily and form better organized and more stable systems than their hydrocarbon counterparts. Owing to these properties, fluorocarbons and fluorinated surfactants have the potential as being used in many applications including the drug delivery, gene transfection, intravascular oxygen transport and other biomedical field [22–26]. In these applications, it is also critical to know the toxicity of the molecules that are utilized. It is reported in the literature that addition of a fluorocarbon chain to the molecular structure reduces both toxicity and hemolytic activity of that molecule compared to their corresponding hydrocarbon counterpart [27–30]. Moreover, it is also demonstrated that cationic surfactants, which are known to be more toxic than nonionic and zwitterionic molecules, having fluorocarbon chain, exhibit lower toxicity toward cells in culture and have a lower hemolytic activity compared to their hydrocarbon analogs as well as being able to self-assemble more easily than their hydrocarbon counterparts which make them good candidates for a gene transfection agent [30]. There is also some evidence that an increasing degree of fluorination reduces the toxicity. Although the fluorinated molecules bring several advantages when they are used, there are discussions in literature on the possible bioaccumulation of fluorocarbons and their effects on living organism. It is acceptable in clinical field to use molecules having fluorocarbon chain less than eight carbons in their structure which makes the use of fluorinated compounds in medical application sustainable [31]. However, the lipophobicity of the fluorocarbon chains, sometimes, limit their utilization. In order to overcome this drawback, usage of fluorocarbon/hydrocarbon hybrid molecules has been proposed [32,33].

Several synthetic surfactants bearing fluorocarbon chains have been synthesized and their interfacial properties as well as the mixing ability with various kinds of lipids have been investigated [34–36]. Especially, the interfacial behaviors of several anionic and nonionic surfactants having different degree of fluorination were presented in the literature [35,36]. For example, in addition to DPPC, the hybrid surfactant (F6PH5PPhNa, sodium phenyl 1-[(4-perfluorohexyl)-phenyl]-1-hexylphosphate or F8PH5PPhNa, sodium phenyl 1-[(4-perfluorooctyl)-phenyl]-1-hexylphosphate) was used as a second component, to form a mixed monolayer at the interface. It was demonstrated that at low surface pressures, there was weak attractive interaction between head groups of these molecules. On the other hand, at high surface pressures, interaction between these two molecules turned out to be repulsive which was resulted from their tail group interaction [35]. Moreover, the maximum surface pressure that could be achieved by this mixture at the maximum compression was lower than the surface pressure that can be achieved when pure DPPC was used. This could be important in some applications [35]. In another study, single-chain (perfluorooctyl) pentanol (F8C5OH) and (perfluorooctyl) pentylphosphocoline (F8C5PC) were used in addition to DPPC to form two-component monolayer system. These hybrid molecules have similar tail group but their hydrophilic head groups are different. It was revealed that the interaction between F8C5PC and DPPC was stronger than that of the interaction between F8C5OH and DPPC because of the hydrophilic choline head group. The calculated excess Gibbs free energy of mixing (ΔG_{ex}) of these two systems were negative which could suggest that these components form mixed monolayer with DPPC and there was attractive interaction between these molecules to a certain extent [36]. On the other hand, their AFM measurements reveal that there was partial miscibility for the F8C5OH/DPPC and phase separation for the F8C5PC/DPPC system. These studies present very important information on the molecular level interactions of fluorinated surfactants and lipid molecules but none of these mixtures have the ability of capturing the behavior of lung surfactant. Recently, it has been demonstrated that addition of F8C11OH to the monolayer having DPPC and He-l 13-5 mimicking polypeptide enhance the properties of the mixtures, although, neither He-l 13-5 nor F8C11OH will not be sufficient to capture the lung surfactant behavior when mixed with DPPC by themselves [37].

In the study presented here, the interfacial behavior of the monolayers formed from the mixtures of the newly designed fluorocarbon–hydrocarbon hybrid cationic surfactant (FHCL, Fig. 1) and DPPC at different mole fractions is evaluated. It is proposed that this new molecule will help to capture some of the selected prop-