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Surface tension and adsorption behavior of mixtures of diacyl glycerol arginine-based surfactants with DPPC and DMPC phospholipids

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ABSTRACT

The binary surface interactions of some novel cationic diacyl glycerol arginine-based surfactants with model phospholipids, which are often used as model membrane components, are studied at 25 °C in aqueous solutions of 0.1 M sodium chloride. The surfactants are 1,2-dimyristoyl-*rac*-glycero-3-O-(N$^{\alpha}$-acetyl-L-arginine) hydrochloride (1414RAc) and 1,2-dilauroyl-*rac*-glycero-3-O-(N$^{\alpha}$-acetyl-L-arginine) hydrochloride (1212RAc), and they are important as potential antimicrobial agents which are biodegradable and with less toxicity than other cationic surfactants. The phospholipids are 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC). The equilibrium and dynamic surface tension of each surfactant, each phospholipid, and some of their binary mixtures are studied using the bubble surfactometry method at constant area or pulsating area conditions. In addition, the surface densities of pure and mixed monolayers of these compounds at the air/water interface are probed with infrared reflection–absorption spectroscopy (IRRAS). Steady state and dynamic surface tension synergism, or antisynergism in one case, and synergistic adsorption effects are detected for the mixed dispersions studied. The enhanced adsorption detected with IRRAS, and the enhanced dynamic and steady state surface tension lowering indicate strong miscibility and net attractive interactions between the compounds in the adsorbed mixed monolayers.

1. Introduction

The economic importance of cationic surfactants was realized early by Domagh [1], who discovered their bacteriostatic properties, leading to many commercial products. Cationic surfactants play important roles as sanitizing and antiseptic agents, germicides, fungicides, and as components in cosmetic formulations. The adaptability of microorganisms, and their tendency to acquire resistance to bacteriocides, makes the development of new antimicrobial agents a recurring challenge. Since antimicrobial compounds introduce risks with respect to environmental and mammalian toxicity, there is a need to develop biodegradable antimicrobial surfactants, the toxicity profiles of which become lower as they degrade with time.

For the past 20 years, new cationic antimicrobial surfactants derived from amino acids have been synthesized [2–4]. The surfactants 1,2-dimyristoyl-*rac*-glycero-3-*O*-(N$^{\alpha}$-acetyl-L-arginine) hydrochloride (1414RAc) and 1,2-dilauroyl-*rac*-glycero-3-*O*-(N$^{\alpha}$-acetyl-L-arginine) hydrochloride (1212RAc), the structures of which are shown in Plate 1, have been synthesized [5–8]. Studies of their physicochemical and biological properties have shown that these compounds combine a satisfactory toxicity profile with a high biodegradability [9,10] similar to that of partial glycerides and lipoamino acids [2,9]. They can be considered analogous to certain phospholipids, such as 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) or 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC); see Plate 1. This is because they exhibit similarly strong surface activities (the ability to decrease the surface tension substantially at low concentrations), low solubilities, and similar phase behavior aggregation properties such as forming with water lamellar lyotropic liquid crystals above a certain temperature [8]. They can be considered to be "soft preservatives" compared to traditional "hard" cationic surfactants, which are less easily biodegradable. Such arginine-based surfactants have acidic guanidinium and N$^{\alpha}$ amino groups the structures of which depend on pH. The acid–base equilibria affect their aggregation behavior [11,12].

Surfactant/phospholipid mixtures are important in many applications, including emulsion and foam stabilization [13,14], lung surfactants [15–17], and the stability of cell membranes [18]. Physicochemical interactions of water-insoluble phospholipid components of cell membranes with soluble or sparingly soluble surfactants in solutions, in dispersions, or at surfaces have been

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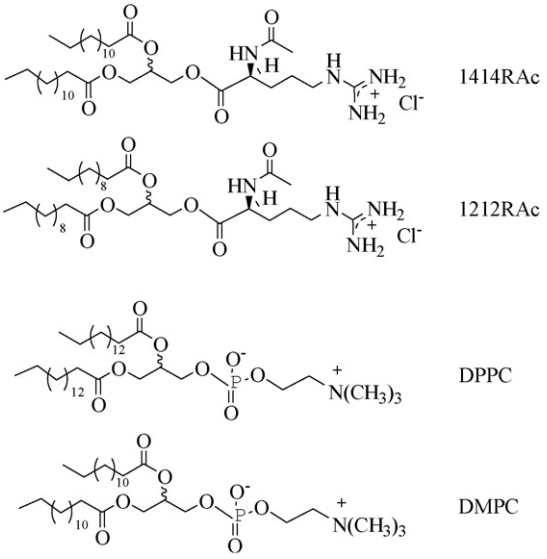

Plate 1. Chemical structures of the diacyl glycerol arginine-based surfactants and the phospholipids tested in this work.

reported [19–25]. Understanding the behavior of model mixed monolayers may help understanding of more complex biological membranes.

In this article we report results of studies of each of the above two cationic surfactants (from now on called "surfactant"), each of the above phospholipids, and several binary mixtures of one surfactant and one phospholipid at the air/water interface using surface tensiometry at constant and pulsating area conditions, and infrared reflection absorption spectroscopy (IRRAS). Mixed monolayers formed by adsorption from solution or dispersion provide different and complementary information from that obtained from studies of spread monolayers. The results also show interesting mixture behavior, including some novel cases of steady and dynamic surface tension synergism and of adsorption synergism [26–29]. The goal is to find out whether the surfactants can mix with the lipids in adsorbed monolayers, and, if so, ideally or non-ideally, and determine possible molecular thermodynamic bases for mechanisms of antimicrobial activity, in which surfactants interact with and affect the behavior of biological membranes.

2. Experimental

2.1. Materials

The diacyl glycerol arginine-based surfactants (1414RAc and 1212RAc) were synthesized with the method described by Pérez et al. [30]. Their purity was higher than 99% by weight. The phospholipids (DPPC and DMPC) were purchased in powder form from Sigma–Aldrich with a purity of 99% by weight. Sodium chloride (>99.8% by weight) was purchased from Fisher Scientific (Springfield, NJ). HPLC-grade hexane was supplied by Panreac, and ethanol (200 proof) was obtained from Pharmco Products, Inc. (Brookfield, CT).

2.2. Preparation of samples

All dispersions were prepared in 0.1 M sodium chloride aqueous solutions on a weight basis. Plastic centrifuge tube containers from Corning® were used, in order to avoid possible ionic contamination from any use of glass. Water was first distilled and then passed through a Millipore four-stage cartridge system, consisting of an organic adsorption column, two mixed ion exchange columns, and an ultrafiltration unit, resulting in a water resistivity of 18 MΩ cm at the exit port. Surfactant/phospholipid mixtures, at surfactant mole fractions X of 0.3, 0.5, and 0.7, and pure surfactant or phospholipid dispersions, were prepared at a total concentration C_T of 500 μM. They all showed a substantial turbidity, indicating they were two-phase dispersions. The solubility of each surfactant is well below 50 μM; that of DPPC and DMPC is well below 1 nM, or essentially "zero" [31]. The dispersions were shaken vigorously by hand at room temperature for 5 min, and then sonicated for 15 min in a sonicator bath (Branson 1200 Ultrasonic cleaner, Branson Cleaning Equipment Co., Shelton, CT). The details of such a preparation protocol are often quite important, affecting the dispersed particle size and the dynamic surface tension behavior [32]. The sonication was done at 50 °C for most systems, and at 25 °C for systems containing only DMPC or 1212RAc, which have melting transition temperatures of about 23 °C. To minimize pH changes induced by prolonged exposure to atmospheric carbon dioxide, and to decrease effects of hydrolysis, the dispersions were examined within 24 h after preparation. Spreading solutions were prepared using hexane/ethanol 9:1 (v/v) with a concentration of 0.7 mM.

2.3. Bubble surfactometer for surface tension measurements (PBS)

A commercial, thermostatted, computer-controlled bubble surfactometer (PBS) from Electronetics Co. (now General Transco, Largo, FL) was used for measuring the dynamic and steady state surface tension of each aqueous surfactant (1414RAc, 1212RAc), phospholipid (DPPC, DMPC), and surfactant/phospholipid mixtures at constant or pulsating area conditions [33–35]. The instrument uses a calibrated pressure transducer for measuring the pressure drop, $\Delta P(t)$, across the air/water interface of a small nearly spherical bubble which is open to the atmosphere. The Laplace–Young equation for spherical interfaces [33],

$$\Delta P(t) = \frac{2\gamma(t)}{R(t)} \quad (1)$$

was used to determine surface tensions $\gamma(t)$ from the measured bubble radius $R(t)$ and the pressure difference $\Delta P(t)$. At constant area conditions, surface tension measurements with R of 0.40 mm were recorded every 50 ms, starting 1 s (the "dead time") after forming a new bubble. At pulsating area conditions, the area of the bubble changes sinusoidally at frequencies ranging from 1 to 100 cycles min^{-1} (rpm), with the radius of the bubble R varying from 0.40 to 0.55 mm, and a ratio of the maximum to minimum area of 1.89. Usually 20 rpm was used in the data reported here. The sample chamber contains 25 μl of liquid. Bulk and surface rheological effects and other dynamic effects are generally unimportant at the conditions used for the validity of Eq. (1), which is strictly valid for spherical interfaces at hydrostatic equilibrium [34,36]. The experiments were conducted at 25 °C. The dispersions were examined the same day they were prepared, to minimize the possible effects of surfactant [30] or phospholipid hydrolysis [37,38].

2.4. Infrared spectroscopy

A Nicolet Protégé 460 Fourier transform infrared spectrometer was used to obtain infrared reflection-absorption (IRRAS) spectra and attenuated total reflection (ATR) spectra at 25 °C. An MCT (mercury–cadmium–telluride) liquid-nitrogen-cooled detector and an external reflection attachment from Graesby Specac Kent, UK, were used. A small Teflon Langmuir trough (with area dimensions of 2.2 cm × 8.1 cm and a liquid volume of ca. 12 ml) was used. The instrument was continuously purged with purified air from a Balston purge gas generator, for reducing water vapor and carbon dioxide in the sample chamber. Approximately 30 min after the closure of the instrument chamber lid, a stable signal was reached.