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Interfacial behavior of Myristic acid in mixtures with DMPC and Cholesterol

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ABSTRACT

Binary mixture monolayers of Myristic acid (MA) with the same length of saturated acyl chain lipid viz 1,2-myristoyl-sn-glycero-3-phosphocholine (DMPC) and Cholesterol (Chol), were investigated under different experimental conditions using Langmuir monolayers (LMs). The interfacial pressure-area (π-A) isotherms, excess molecular area, excess free energy and fluorescence microscopy (FM) images were recorded at the air/water interface. Monolayers of both systems (e.g. MA/DMPC, MA/Chol) reach the closest acyl hydrophobic chain packing in the range $0.20 < x_{MA} < 0.70$. Thermodynamic analysis indicates miscibility of the binary mixtures when spread at the air/water interface with negative deviation from the ideal behavior. Morphological features of MA/DMPC systems were found to depend strongly on MA mole fraction and pressures by showing two extreme minima in Gibbs free energy of mixing, while MA/Chol systems showed only an effective condensing effect at $x_{MA} = 0.90$. In the whole range of compositions studied here, the liquid-expanded (LE) to liquid-condensed (LC) phase transition occurs at increasing x_{AM} as it accomplished by a huge increase in the inverse compressibility modulus. FM observations confirmed the phase-transition and condensing effects of both mixture monolayers as evidenced by Gibbs free energy of mixing in a limited range of compositions.

1. Introduction

Pseudo-physiological Langmuir monolayers (LMs) are frequently used to establish the necessity required to mimic their counterpart of a single leaflet in eukaryote cell membrane [1,2]. In the past decades, many research interests in phospholipids membranes were based on this fact, therefore mimicking the outer leaflet of natural cell membrane by a simple model known as LM at the air/water interface was found to be beneficial [2–4]. It is well established that lipid-bilayer encapsulated within the cell membrane isolates the inner compartments from outer environment. Many important cellular processes which can take place at the outer membrane interface are stimulated by external agents such as proteins, ions and other soluble macromolecules [5,6]. It is widely accepted that many of these important phenomena that occur at the sub-cellular level, in principle, can be '*in vitro*' experimentally modeled with LMs at the air/water interface in a control manner [4,7,8].

LMs at the air/water interface are 2D structures that have been intensively used to elucidate the interfacial and elastic properties of natural cell membranes under a simplified and controlled physicochemical environment. However, this simple technique has many advantages when compared with the methods employed in 3D objects such as vesicles, emulsions and micelles. In addition, monolayers' interfacial structure and phases at interfaces can be visualized at micro- and nanoscale by various optical techniques such as fluorescence microscopy (FM) [9], Brewster angle microscopy (BAM) [10], and atomic force microscopy (AFM) [11]. Thus, many research groups have employed the LM technique to understand what is happening at the sub-cellular level when interactions are taking place between membrane-proteins, peptides and others stimulus [4,6].

Fatty acids are amphiphiles that are widely found in nature either in plants or animals. Pure forms of these compounds and/or their mixtures have important applications in medicine and show several biological functions. Furthermore, the physical properties of these compounds when mixed with phospholipids bear in its nature a rich variety of phases which are closely related to the molecular packing and the structure of cell membrane. The 2D study of these mixtures at the air/water interface is very

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informative in subjects such as chain packing, phase transition and composition of membranes. The structural properties of monomolecular films have been thoroughly studied by LM technique to gain insights about their natural membrane counterpart [2,12,13].

In the present work, our attention is focused on the effect of polar headgroups charge and the hydrophobic interactions on the monolayer elasticity and miscibility. Thus, MA (also known as tetradecanoic acid) was selected as one of the cell membrane components [14]. This is due to the fact that MA has a sufficiently high hydrophobicity to be incorporated into the hydrophobic core of plasma cell membrane in higher eukaryotic organisms. Another essential role of the MA emerging from the fact that some proteins and peptides across cell membrane are translocated by acylating their *N*-terminal end with fatty acids like MA and palmitic (PA) acids [15,16]. For example, Lipopetides are known to be acylated with MA or PA when imposed externally to the cell membrane, thus modulating biologically important activities across the membrane, whereas their non-acylated forms are unable to trigger any biological response [17]. Hence, it seems that acylation with these two fatty acids promotes translocation of the anchored proteins across the cell membrane [16]. This process is terminated as myristoylation or palmotoylation which in turn target and anchor peptides and proteins with specific biological functions within the cell interior [15]. Consequently, acylation appears to be vital for protein-membrane attachment especially for proteins with hydrophobic mismatch with respect to the lipid bilayer hydrophobic core, thus, facilitating membrane anchoring process to overcome this mismatch [18,19]. Such a hydrophobic modification has been shown to play a key role in protein-protein association, protein targeting, and protein binding to membranes [17]. It's worth mentioning here, that besides MA (C14:0) other fatty acids (*i.e.*, C12:0 C14:1 C14:2 C16:0) may participate in the acylation process [19].

MA is a single acyl carbon chain (C14:0) molecule which forms a LM with a rich phase behavior at the air/water interface [20]. The MA monolayer with temperature-sensitive interfacial behavior has been widely investigated using various methods [21], and its phase diagram is well-known to researchers who are employing the LM technique [22]. Accordingly, the use of MA as one of the binary components in mixed systems allows the researchers to interpret the interfacial properties induced by other lipids [23]. Therefore, MA/DMPC and MA/Chol monolayers were prepared to study their role on membrane properties at the air/water interface. Owing to the fact that these mixed monolayers are too simple to mimic the real cell membrane, but nevertheless it gives an over simplified idea about the molecular interactions and/or organization of these molecules at cellular level without invoking possible myristoylation of a specific protein. The DMPC lipid is a zwitterionic amphiphilic molecule with saturated double bond, containing 14 carbon atoms each, with a well-known phase behavior at the air/water interface [24]. It is one of the most commonly used lipids in monolayer studies due to its presence in mammalian membranes. In addition, both MA and DMPC molecules have essentially the same hydrophobic chain length. The headgroups of DMPC molecule possess dipole moment with an overall orientation greatly contributes to electrostatic interactions with the MA molecule headgroups across the monolayer interface at which they are partially immersed in water [25,26]. On the other hand, Chol as well as other sterols are known to influence the conformational order of the lipid acyl carbon chains and membrane fluidity [27]. Chol is also the main sterol component with a maximum of 30 mol% of the biological membranes composition [1]. Furthermore, sterols regulate the membrane bilayer hydrophobic thickness which is usually attributed to membrane-proteins interactions. Sterols, in particular Chol, have cohesive forces toward saturated lipids by influencing sterol-fatty acids lateral headgroups packing and consequently membrane properties forming liquid-ordered phase which co-exists with liquid-disorder phases [28]. In general, Chol molecule orients itself with its planer rigid ring in proximity of the phospholipid or the fatty acid headgroups near the outermost membrane hydrophilic interface, and aligns its long molecular axis parallel to acyl chains within the hydrophobic core of the cell membrane [29,30].

The aims of this study are to clarify the interfacial elasticity of MA/DMPC and MA/Chol monolayer mixtures, the miscibility of MA with DMPC or Chol, and its LC-microdomains. In an attempt to find out how these liquid-condensed microdomains are achieved in these binary mixtures, the single chain fatty acid MA is mixed with the same acyl chain/lipid which may structurally mimic the driving force to form these LC-phases at the air/water interface. These simple experiments may also shed some light on the ability of acyl chains, without peptides or proteins acylation, in a phospholipids and cholesterol when mixed with single chain fatty acids to act independently in condensed microdomains formation. This process in turn may not require the glycerol backbone chains' headgroups to form such domains [2,7]. In fact, these microdomains are primarily originated from Simons and Ikonen [31] idea of lipid domains or rafts in cellular mammalian membrane. They have suggested to selectively include or exclude membrane proteins while important processes occurring within the cell membrane like transport or signal transduction [3,8]. Interfacial pressure-molar area (π-A) isotherms and the corresponding thermodynamic properties of the mixed monolayers, were measured at the pure air/water interface at T = 25 °C. The elastic properties of the binary mixtures were measured using the inverse-compressibility modulus extracted from π-A isotherms. The phase behavior was examined using the Gibbs additive rule as well as the Gibbs free energy of mixing. Furthermore, clarifying the phase co-existence regions and the microdomains phase morphology of the binary mixed monolayers were investigated by FM technique.

2. Experimental and theoretical background

2.1. Materials

1,2-myristoyl-sn-glycero-3-phosphocholine (DMPC) was purchased as lyophilized powder from Avanti Polar Lipids (Alabaster, AL, USA). Myristic acid (MA), Cholesterol (Chol) and spreading solvents (i.e., chloroform and methanol) were purchased from Sigma Aldrich (Taufkirchen, Germany). All chemicals were of highest available purity and used without further purification. The MilliQ water (Millipore UV plus system, produce water resistivity $\rho > 18.2$ MΩ.cm at T = 25 °C) was used in all the preparations. Stock solutions ($c = 0.10 \pm 0.01$ mgml^{-1}) were prepared by dissolving DMPC, Chol or MA in chloroform. The obtained solutions were then used to prepare solutions of the desired mixture ratios. Specific amounts of lipid were then mixed with MA solution to form lipid/MA mixtures with varying molar fractions of x_{MA}.

The experiments described below focus on the effect of these two molecules on the MA- monolayer's compressibility and morphology at the air/water interface, and their effect on the thermodynamics of the mixed monolayer at different MA concentrations. Solutions with different bulk concentrations were used because the lift-off areas depended on the mixtures ratios (see Section 3.1). Brewster angle microscope (BAM) enables both visualization of the film texture and estimation of the monolayer thickness at different stages of compression. The film thickness derived from the relative intensity measurements of BAM was estimated to be between 1.75 ± 0.02 nm and 2.10 ± 0.02 nm for LE and LC phases respectively [32]. With the help of this method, the monolayer thickness