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Lyso-phosphatidylcholines in Langmuir monolayers – Influence of chain length on physicochemical characteristics of single-chained lipids

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ABSTRACT

Single-chained phospholipids constitute a class of membrane components found in normal cells in relatively low concentration; however, these group of compounds are known owing to their broad physiological activities. Despite that the knowledge concerning fundamental physicochemical properties of lyso-lipids is very limited and in contrast to double-chained phospholipids there is an obvious deficiency of studies focused on correlation between their amphipathic character and film-forming properties with biological activities. In the present paper we have attempted to explain the main issues regarding the characteristics of lyso-PCs in monolayers at the air/aqueous interface.

Our results show that all the investigated phospholipids differing in the length of hydrophobic chain: C_{18}lyso-PC, C_{22}lyso-PC and C_{24}lyso-PC form stable Langmuir monolayers of a relatively low degree of condensation. It was found that during compression the investigated monolayers significantly change their organization at the interface which is strongly connected with temperature of the subphase. The application of X-ray reflectivity confirmed that the bulky choline head-groups in molecules of lyso-PCs are strongly penetrated by water molecules, while the hydrophobic chains are significantly tilted from the surface normal. The obtained results show that the phase transitions observed in the course of the registered isotherms result from decrease in immersion of molecules in the subphase as well as from the decrease in hydrating water molecules. On the basis of GIXD experiments it turned out that in the monolayers of C_{22}lyso-PC and C_{24}lyso-PC at higher surface pressures (>20 mN/m) small fractions of periodically ordered phase appear at the interface. For the monolayer of C_{24}lyso-PC in the periodically ordered fraction the untilted (U) to tilted (t) phase transition was found.

1. Introduction

Physicochemical characteristics of biological membrane phospholipids for many years has drawn special attention of scientists and continuingly is a subject of extensive studies performed both on cell cultures in vitro as well as in a variety of artificial (model) systems. The driving force for such investigations is a need to understand the complicated processes that govern cells, in which the lipid membrane with its multiple functions and, in large extent still unrecognized properties, fulfill a significant role. Among suitable methods which are widely applied to study properties of membrane lipids, the Langmuir monolayer technique offers many incontestable advantages. This methodology enables the generations and study of monomolecular films of an amphiphile at the air/water interface, therefore can be adapted as a simple but well-designed model of a single bilayer leaflet [1,2]. In this technique both the experimental conditions as well as the composition and architecture of membrane-mimicking films can be easily adjusted. Among extensively studied membrane lipids special place belongs to phospholipids which comprise the main class of natural components in bilayers. These amphipathic compounds differ in their chemical structures both in terms of polar head-groups as well as hydrophobic chains [3]. Generally, the most typical representative of membrane phospholipid, also from the statistical point of view, is phosphatidylcholine bearing in molecular structure two acyl chains of 16 or 18 carbon atoms, among which one is fully saturated, while the other possess one double bond [4]. Of course, biological membranes vary significantly as regards lipids compositions which is caused, e.g. by different functions of particular organelles, cells and tissues [5,6]. It is also well known that phospholipids content in membrane may change in some inflammatory processes and diseases, like, e.g. diabetes [7] or cancer [8,9]. Despite their important structural function, double-chained phospholipids occurring in membranes in high percentage of total lipid composition serve also as a specific reservoir for compounds which are the products of enzymes action on these phospholipids. The important examples here are the processes catalyzed by

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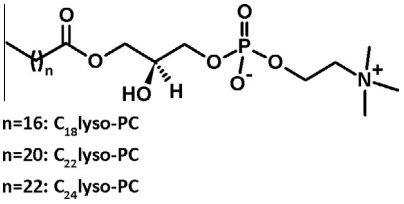
phospholipase A2 (PLA2) – the enzyme which hydrolyzes the sn-2 ester linkage in molecule of diacyl phospholipids releasing two products: lyso-phospholipid and free fatty acid [10].

Lyso-phospholipids (lyso-PLs) are compounds found in cellular membrane in relatively low concentration, that is averagely of 0.5–6% of total lipid mass [11]; however, their content may increase in the case of some diseases like atherosclerosis, hyperlipidemia, arrhythmia or inflammatory processes [12]. The elevated concentrations of lyso-lipids in membranes cause significant alteration of their properties and structure. It was found that abnormally high concentration of lyso-compounds in lipid bilayers may lead to dysfunction of some membrane proteins, e.g. ion channels [13,14] and in extreme situation cause cell lysis [15]. On the other hand, there are also results of studies suggesting that concentration of lyso-PLs in cancer patients decrease [16]. In contrast to cell membranes, lyso-phosphatidylcholine is one of a major lipid component found in oxidized low density lipoproteins, oxLDL (~40% of total lipid content) and in VLDL (very low density lipoprotein) [17]. Moreover, lyso-PC modulates the properties and functions of these lipoproteins, which is a reason why this molecule activity is frequently connected with cardiovascular diseases, especially atherosclerosis [10,18].

Lyso-phospholipids are implicated in a broad range of other important cellular processes including signal transduction, gene transcription, mitogenesis and vascular smooth muscle relaxation [19–21]. Moreover, lyso-PCs were found to be responsible for modulation of the intracellular calcium concentration [10,22] and are also recognized to be a chemotactic factor for monocytes [23]. Despite their broad functions, the mechanisms of activities displaying by lyso-phospholipids at the level of biological membrane still remain uncertain.

It is also worth mentioning that lyso-lipids fulfill other very important role in membrane architecture, namely they are known to be inhibitors of biological processes connected with membrane fusion, like for example endo- and exocytosis, fertilization or some viral infections [11,24]. The main reason of specificity of lyso-PLs in this context is the characteristic chemical structure and in consequence the spatial arrangement of their molecules in natural bilayers. It is known that single-chained phospholipids possessing in their molecular structure bulky head-group and only one hydrophobic chain are often classified to the group of 'inverted cone-shaped lipids' or 'micelle-forming lipids' [25,26]. The direct reason of this fact is that the cross sectional area of the head-group is significantly larger than that of the single hydrocarbon tail. Such specific shape causes that molecules of lyso-PLs tend to spontaneously assemble into bent structures (e.g. micelles) rather than into flat layers. Lyso-PLs, especially these possessing large polar head-group (lyso-PCs rather than lyso-PEs and lyso-PA) have high positive spontaneous curvature in contrast to diacyl phospholipids of a negative curvature [27]. This means that incorporation of lyso-PL molecules into membrane changes important mechanical properties of lipid bilayer, like tension or stress [28]. Of course, the effect of lyso-PLs on membrane curvature and in consequence fusion processes depends on their distribution between inner and outer leaflet [27]. For example, in membranes of human erythrocytes lyso-PC are present almost exclusively in the outer layer [29].

In contrast to their double-chained precursors, studies concerning physicochemical characteristic of lyso-phospholipids are rather rare. For example, in scientific literature there is evident lack of data as regards the fundamental properties of monomolecular films formed by lyso-lipids. To fulfill this gap we undertaken studies focused on the behavior of three representatives of lyso-phosphatidylcholines in monolayers at the air/water interface. For our investigation we selected the following single-chained compounds: C_{18}lyso-PC, C_{22}lyso-PC and C_{24}lyso-PC (Scheme 1).



Scheme 1. Molecular structures of the investigated lyso-phospholipids: C_{18}lyso-PC, C_{22}lyso-PC and C_{24}lyso-PC.

As it can be seen the molecules of the above presented lipids differ in the length of hydrophobic acyl chain, whereas the polar head-group is the same. It should be mentioned here that since C_{18}lyso-PC is one of the dominating species of lyso-phosphatidylcholines found in cellular membranes, the other two derivatives having longer hydrophobic chains should rather be treated as traces in natural samples. On the other hand, because of high physiological activity of lyso-PLs, their exogenous administration could be considered in pharmacological treatment. The examples of single-chained compounds having long hydrophobic part (C_{22}) can be found among molecules possessing promising pharmacological potential against cancer [30]. It is evident that increase in the hydrocarbon chain length affects significantly the main properties of these compounds, starting from their solubility, trough ability to modify membrane fluidity and permeability, and ending at molecular recognition by membrane receptors.

In our studies we have applied the Langmuir monolayer technique to characterize interfacial properties of lyso-PCs differing in the length of hydrocarbon chains: C_{18}lyso-PC, C_{22}lyso-PC and C_{24}lyso-PC. The undertaken experiments based on elementary monolayers characterization in terms of surface pressure (π) – molecular area (A) isotherms as well as stability tests. We have also applied Brewster angle microscopy (BAM) in order to directly visualize the morphology of the investigated films. The mentioned studies have been complemented with techniques based on synchrotron X-ray radiation scattering, that is X-ray reflectivity (XR) and grazing incidence X-ray diffraction (GIXD).

2. Experimental

2.1. Materials

The investigated single-chained phosphatidylcholines, namely C_{18}lyso-PC (1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine), C_{22}lyso-PC (1-behenoyl-2-hydroxy-*sn*-glycero-3-phosphocholine) and C_{24}lyso-PC (1-lignoceroyl-2-hydroxy-sn-glycero-3-phosphocholine) of the highest purity available in stock (>99%) were purchased from Avanti Polar Lipids and used without further purification. Spreading solution of the lipids of the concentration close to 0.25 mg/ml were prepared in chloroform/methanol 9/1 (v/v) mixture. Chloroform of spectroscopic purity (99.9% stabilized by ethanol) as well as methanol (99.9% were provided by Sigma–Aldrich. In all experiments on Langmuir trough ultrapure water of the resistivity $\geqslant$18.2 MΩ cm from MilliQ system was applied as a subphase.

3. Methods

3.1. Langmuir experiments

In routine experiments, π–A isotherms and stability measurements were recorded with the NIMA (Coventry, UK) Langmuir