Effects of hydrocarbon chains saturation degree on molecular interaction between phospholipids and cholesterol in mixed monolayers

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Monolayers of three mixed systems of cholesterol (Chol) with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-choline (POPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) were investigated at the air/water interface. Based on the isotherms of monolayers, the molecular interaction and miscibility have been evaluated in terms of the excess mean molecular area ($\Delta A_{\text{ex}}$) and excess Gibbs free energy ($\Delta G_{\text{ex}}$). The excess molecular area ($\Delta A_{\text{ex}}$) of DOPC/Chol monolayers was negative at $X_{\text{Chol}} = 0.2$ and 0.6, indicating it was attractive interaction. The repulsive interaction was observed at $X_{\text{Chol}} = 0.4$. In the range of $X_{\text{Chol}} = 0.8$, the $\Delta A_{\text{ex}}$ was negative at the surface pressure of 5, 10 and 15 mN/m, positive at 20, 25 and 30 mN/m. In the POPC/Chol monolayers, the value of $\Delta A_{\text{ex}}$ was negative for all the studied mixed monolayers except for 30 mN/m at $X_{\text{Chol}} = 0.8$. The thermodynamic behavior in DPPC/Chol monolayers was more complex. The $\Delta A_{\text{ex}}$ was negative only at $X_{\text{Chol}} = 0.2$ and lower pressure at $X_{\text{Chol}} = 0.4$, and 0.6. The stability of mixed monolayers was assessed by the parameter of excess Gibbs energy. For the three binary monolayers, the maximum appeared at the points of $X_{\text{Chol}} = 0.6$, $X_{\text{Chol}} = 0.4$ and $X_{\text{Chol}} = 0.2$ for DOPC/Chol, POPC/Chol and DPPC/Chol, respectively. The results of the analysis for elasticity modulus showed the saturated DPPC monolayer was more elastic than unsaturated PCs. The different unsaturation chains of PCs could determine the different compressibility behavior. These results can help to understand the thermodynamic behavior of membranes made of mixtures of PCs with different chains and cholesterol.

Keywords: Attractive and repulsive, Compressibility, Gibbs energy, Excess molecular area, Monolayers

Phospholipids and cholesterol are important structural and functional components of biological membranes. The understanding of the assembly and deposition of these molecules is important for biomembrane. PCs are also often used as the main phospholipids to study the dynamic character of the membrane in biophysical studies and have attracted a great attention because they are one of the most abundant ingredients in cell membranes. Several characteristics of the lipid molecules govern their phase and packing structure behavior in the lipid membranes, including their chemical structure, molecular charge, and stereochemistry. In cell membrane, the interaction between Chol and other lipids has been studied by various physicochemical methods.

Langmuir-Blodgett (LB) technique is an ideal method to study the dynamic characteristic of monolayers and bilayers, in which the molecular interactions have been studied extensively. The isotherms of monolayers were used to investigate the interfacial properties and the molecular interaction and miscibility range of components. The objective of this study was to compare the effect of varying the chain saturation degree of lipids on the mixture with DOPC, POPC, DPPC and cholesterol at the air-water interface by using surface pressure ($\pi$)-area ($A$) measurements. Our results give rise to a better understanding of the interaction between PCs and cholesterol at a molecular level.

Materials and Methods

Preparation of mixed monolayers

1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) were purchased from Avanti Polar Lipids (Alabaster, AL) and used as received. The preparation of the monolayers was performed in a computer-controlled device (Minitrough; KSV, Helsinki, Finland). For all the experiments, the subphase was water (18.2 MΩ cm), obtained from a Millipore purification system.

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The lipids were dissolved in chloroform-methanol (3:1, v/v) to a final concentration of 0.1 mg/mL. Each 250 μL of the sample solution was spread on the subphase with a Hamilton microsyringe. About 15 min later, compressed the monolayer at a constant rate of 10 mm/min. The subphase temperature was maintained constant at 20 ± 0.5°C by circulating water through the base plate on which the trough was mounted.

Isotherms analysis

Based on the analysis of π–A isotherms, for ideal mixing the mean area per molecule $A_{12,\text{ideal}}$ is defined as

$$A_{12,\text{ideal}} = (A_1)\pi X_1 + (A_2)\pi X_2$$  \hspace{1cm} (1)

Where $A_1$, $A_2$ are the molecular area of a single component at a definite surface pressure and $X_1$, $X_2$ are the mole fractions of components 1 and 2 in a mixed film.

The excess area $\Delta A_{\text{ex}}$ per molecule can be calculated by the equation

$$\Delta A_{\text{ex}} = A_{12,\text{exp}} - A_{12,\text{ideal}}$$  \hspace{1cm} (2)

Where $A_{12,\text{exp}}$ was the values obtained by experiments.

The interaction between cholesterol and lipids in mixed monolayers and its thermodynamic stability can be investigated by means of the excess Gibbs energy of mixing $\Delta G_{\text{ex}}$. The Gibbs energy change with mixing of component 1 and 2, $\Delta G_{\text{mix}}$, for a real mixed system, is considered to be a sum of ideal and excess Gibbs energy changes as

$$\Delta G_{\text{mix}} = \Delta G_{\text{ideal}} + \Delta G_{\text{ex}}$$  \hspace{1cm} (3)

For ideal mixing the Gibbs energy change involves only the entropy term as

$$\Delta G_{\text{ideal}} = RT(X_1 \ln X_1 + X_2 \ln X_2)$$  \hspace{1cm} (4)

Where $X_i$ (i = 1, 2) denotes mole fraction in the mixture and R is the gas constant times Kelvin temperature. Further, the excess Gibbs energy can be expressed as:

$$\Delta G_{\text{ex}} = \int_\pi [A_{12,\text{exp}} - (X_1 A_1 + X_2 A_2)] d\pi$$  \hspace{1cm} (5)

Where $A_{12,\text{exp}}$, $A_1$, and $A_2$ represent the area of mixed system and respective areas of components 1 and 2, and $\pi$ is the surface pressure of monolayer. If the monolayer is an ideally mixed one, $\Delta G_{\text{ex}}$ should be zero.$^{12}$

For analysis of the properties of monolayer films, compressibility $C_s$ or elasticity $C_{s}^{-1}$ may be used as an important parameter.$^{13}$ The values can be obtained as follows

$$C_s = -\frac{1}{A} \left( \frac{\partial A}{\partial \pi} \right)_T$$  \hspace{1cm} (6)

Where $A$ and $\pi$ are the mean molecular area and surface pressure, respectively.

Results and Discussion

π-A compression isotherms at discrete mole fractions

The surface pressure-area (π-A) isotherms of DOPC/Chol, POPC/Chol, and DPPC/Chol mixed respectively monolayers at various mole fractions are shown in Fig. 1. Isotherms of pure DOPC, POPC, DPPC, and cholesterol were consistent with others in the literature.$^{14,15}$ The monolayers of pure DOPC and POPC were always in the liquid expanded (LE) phase and showed a collapse surface pressure of 46.5 ± 0.6 mN/m and 47.1 ± 0.5 mN/m. In turn, the monolayer of DPPC is in LE up to 5.2 ± 0.2 mN/m where it exhibits a characteristic phase transition between the disordered liquid-expanded (LE) and the ordered liquid-condensed (LC) phases. The collapse was observed at 56.1 ± 0.4 mN/m. For cholesterol, the practically linear increase of surface pressure up to point of collapse beyond 39.5 Å²/molecule indicates a closely packed monolayer structure, which exists as a solid-phase during the compression.

When cholesterol was incorporated into the DOPC monolayers, all the isotherms of the mixed system appear in the order of increase in mole fraction between those of both single systems (Fig. 1A). The results of POPC/Chol isotherms became more complex when the contents of cholesterol at $X_{\text{chol}} = 0.4, 0.6, 0.8$ for the surface pressure over 25 mN/m (Fig. 1B). The isotherms of DPPC/Chol mixed monolayers overlapped for the liquid condensed phase ($\pi >$10 mN/m) (Fig. 1C). The DOPC, POPC, and DPPC have the same head group, but with different chains. It can be concluded that the chains have affected the molecular interaction behavior of mixed monolayers.
Miscibility analysis of the mixed monolayers

The binary miscibility can be assessed by mean molecular areas. When the mixed monolayer components are miscible, the mixed film shows nonideal behavior, resulting from deviation from linearity. On the other hand, if two components are immiscible or ideally miscible, the $A_{12,\text{exp}}$ is straight line. In Fig. 2 A-C, the $A_{12,\text{exp}}$ for the DOPC/Chol, POPC/Chol and DPPC/Chol mixed systems were plotted against $X_{\text{Chol}}$ at surface pressures $\pi = 5, 10, 15, 20, 25$ and $30 \text{mNm}^{-1}$. As it can be seen, there is no linearity between the mean molecular areas available and the mole fraction of cholesterol for all the three binary systems. According to the data, it is concluded the investigated mixed lipids are miscible and interact in mixed monolayer in a whole range of cholesterol mole fraction and surface pressures.

The observed mixing nonideality is further analyzed by excess area $\Delta A_{\text{ex}}$. The $\Delta A_{\text{ex}}$ can be calculated by the equation 2. When the two components are immiscible or form an ideal mixture, $\Delta A_{\text{ex}}$ equals zero. The negative deviations from ideality suggest attractive interaction between molecules. Furthermore, positive deviation from linearity indicates repelling interaction between the two compounds while negative deviation means induced condensation of the film. The excess molecular area was negative at $X_{\text{chol}} = 0.2, 0.6$ for all the selected pressures and there was an attractive interaction between DOPC and cholesterol from the Fig. 2D. The value was positive $X_{\text{chol}} = 0.4$ and it was repulsive interaction. For $X_{\text{chol}} = 0.8$, the $\Delta A_{\text{ex}}$ were negative for $5, 10, 15 \text{mN/m}$, positive for $20, 25, 30 \text{mN/m}$. For the POPC/Chol system, the excess molecular area values was negative for all the cholesterol molar ratio and selected the surface pressures except for $30 \text{mN/m}$ at $X_{\text{chol}} = 0.8$ (Fig. 2E), this suggests a strong attractive interaction between
POPC and cholesterol as condensing effect. In the system of DPPC/Chol system, there was remarkable negative only at X_{chol} = 0.2. For other ratios, the excess molecular area values were positive for all the surface pressures except 5mN/m. The molecules at the lower pressure of mixed monolayers were possible so far away from each other that the interaction was the attractive force. The attractive force gradually reduced with the surface pressure increases until the force became repulsive seen the Fig. 2F.

Stability analysis of the mixed monolayers
The stability of monolayers was assessed by the parameter of excess Gibbs energy (ΔG_ex). Fig. 3 shows the ΔG_ex as a function of mole fraction of the cholesterol at certain pressures, namely: π = 5, 10, 15, 20, 25 and 30 mN/m. ΔG_ex was calculated based on Eq. (5). When the values of the excess Gibbs energy are negative, it is indicated that the interactions between molecules are more attractive or less repulsive as compared to those in their respective one-component monolayers. For the three binary systems, the lowest values of the ΔG_ex are observed at the position of X_{chol} = 0.6, X_{chol} = 0.4 and X_{chol} = 0.2 for DOPC/Chol, POPC/Chol, and DPPC/Chol, respectively (Fig. 3) and the mixed monolayers were more stable at these ratios of cholesterol.

Compressibility analysis of the mixed monolayers
The influence of cholesterol on the physical state of lipids monolayer can be verified based on the analysis of elasticity modulus (C_s^{-1}) values for mixed films. In Fig. 4 are presented C_s^{-1} vs. surface pressure plots for mixtures of cholesterol at different pressures with DOPC (A), POPC (B), and DPPC (C). The addition of cholesterol into the PCs films causes an increase of C_s^{-1} values, indicating that the mixed films to be more rigid and closely packed. With the increase in cholesterol content in each lipid film, the C_s^{-1} value increase in the order of mole fraction. The maximum of the C_s^{-1} curve corresponds to the maximum elasticity. For the single components, the maximum magnitude of elasticity is 99 mN/m, 97 mN/m, 221 mN/m and 416 mN/m for DOPC, POPC, DPPC, and cholesterol respectively. From the data, the monolayer of cholesterol has a marked elasticity due to the rigid molecular structure. The saturated DPPC monolayer has more elastic than DOPC and POPC. The different unsaturation chains of PCs could determine the different compressibility behavior.

Conclusion
In this work, the influence of fatty acids chains on the model cholesterol/PCs monolayers was studied at the air-water interface. The investigated PCs possess
different saturation degree of the hydrocarbon chains. The thermodynamic analysis indicates miscibility for the POPC/Chol with negative deviation from the ideal behavior, as evidenced by the results of the excess area and excess Gibbs energy analysis. The values of the $C_s^{-1}$ for binary DPPC/Chol mixtures are higher than DOPC/Chol, POPC/Chol monolayer, it is clear that saturated fatty acid makes model membrane more rigid. It will be preliminary to study also the interaction between the phospholipids with different functional groups and cholesterol. This study is in progress and will be the subject of a forthcoming paper.

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