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Thermodynamic Aspects of Cholesterol Effect on Properties of Phospholipid Monolayers: Langmuir and Langmuir–Blodgett Monolayer Study

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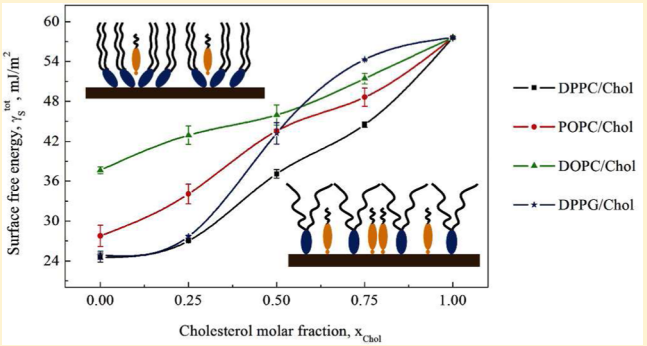
ABSTRACT: Cholesterol is an important component of lipid rafts in mammalian cell membranes. Studies of phospholipid monolayers containing cholesterol provide insight into the role of cholesterol in regulating the properties of animal cells, raft stability, and organization. In this contribution, a study of the characteristics of binary Langmuir monolayers consisting of phospholipids, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) (DPPG), and cholesterol (Chol), was conducted on the basis of the surface pressure–area per molecule (π–A) isotherms. Analysis of the results obtained provided information on the mean molecular area, the excess Gibbs energy of mixing, and condensation in the monolayer. The mixed monolayers were also deposited onto the mica plates and investigated by the contact angle measurements of water, formamide, and diiodomethane. The contact angles allowed calculating surface free energy of the films from the van Oss et al. approach. It was found that cholesterol determines the molecular packing and ordering of the monolayers closely connected with the kind of phospholipid. This is reflected in the values of surface free energy of the model membranes. From the thermodynamic analysis of phospholipid/cholesterol/liquid interactions, one may draw conclusions about the most favorable composition (stoichiometry) of the binary film which is especially important in view of the lipid rafts formation.



1. INTRODUCTION

Cholesterol (Chol) is an important lipid of mammalian cells which plays a crucial role in the lateral organization of lipids in membranes. Its influence on fluidity and order–disorder states of the bilayer has been the subject of intense investigations over the past decades.[1−3] One unique property of cholesterol is its condensing effect on phospholipids in mixtures. It was first discovered in the monolayer studies at the air–water interface where the area per phospholipid molecule was found to be much lower in the presence of cholesterol.[4−7] Different conceptual models have been proposed to explain the nonideal phospholipid/cholesterol interactions. The examples include the condensed-complexes model,[7−10] the superlattice model,[11−13] the umbrella model,[14−16] and others.[17] In these models the considered principal mechanisms leading to condensing are very different. The principles of models are briefly presented in Table 1.

The formation of the complexes may have relevance to biological membranes, as one of the recent advances in the field of membrane biophysics is the existence of specialized regions in cell membranes referred to as 'lipid rafts'. Rafts are considered to be submicroscopic domains enriched in cholesterol, sphingolipids, and certain lipid-anchored proteins but depleted of unsaturated phospholipids.[2,18,19] These laterally segregated domains are found to be important for a variety of cellular functions, such as signaling pathways,[20] as well as for playing a significant role in HIV, Alzheimer's, and prion diseases.[1] The main driving force for the formation of rafts or domains in the membrane is lipid–lipid interactions which depend on the physicochemical properties of lipids,[21] and these

Table 1. Models of Phospholipid/Cholesterol Interactions

model	principal mechanism of condensation	references
condensed complex model	formation of ordered condensed complexes of the fixed stoichiometry	7–10
superlattice model	regular lateral distribution of cholesterol in the phospholipid matrix	11–13
umbrella model	association of cholesterol with certain phospholipid molecules whose polar headgroups shield cholesterol from interactions with water	14–16
other	formation of alloy-like mixed domains in which the vertical position of cholesterol with respect to phospholipid depends on its concentration	17

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interactions influence the organization, packing, and ordering of molecules in the membranes. However, there is still ambiguity concerning the nature of these domains with respect to their size, function, and composition.

Introduction of cholesterol into the phospholipid monolayer can lead to its modification with respect to hydrophilic–hydrophobic properties, which are closely related to the interactions between various components of the system. In this study, characterization of the condensing effect and the interactions occurring in the mixed phospholipid/cholesterol monolayers at the air–water interface was made through analysis of the surface pressure–area per molecule (π–A) isotherms. The isotherms provided information on the mean molecular area occupied by one molecule in the monolayer, the film compression modulus and the excess Gibbs energy of mixing. On the other hand, the monolayers of analogous composition, supported on mica, were investigated by measurements of the contact angles of water, formamide, and diiodomethane. This allowed calculation of their surface free energy according to the theoretical approach developed by van Oss et al. (Lifshitz–van der Waals Acid–Base Model, LWAB).[22−24] In this way, it was possible to correlate the monolayers' properties at the air–water interface and those determined for the solid-supported monolayers with the monolayer composition.

2. EXPERIMENTAL SECTION

Materials. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, 99%), 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine (POPC, 99%), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC, 99%) (DOPC), 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-1-glycerol (DPPG, 99%), and cholesterol (Chol, 99%) were purchased from Sigma and used without further purification. Water from the Milli-Q Plus system (resistivity 18.2 MΩcm) was used both as a subphase for the Langmuir monolayers and as a probe liquid in the contact angle measurements. The other probe liquids were formamide (98%, Aldrich) and diiodomethane (99%, Aldrich). Chloroform (p.a., POCH S.A., Poland) and ethanol (96%, p.a, POCH S.A., Poland) for lipid dissolving were employed as received. Freshly cleaved mica plates (Continental Trade, Poland) of 38 mm × 26 mm × 1 mm size were applied as a solid support.

Determination and Analysis of π–A Isotherms. The surface pressure–molecular area (π–A) isotherms were obtained with a computer-controlled KSV standard-trough (KSV Instruments Ltd., Finland) equipped with two symmetrical barriers and a platinum Wilhelmy plate for the surface tension measurements. The temperature of the subphase was maintained at fixed 20 ± 1 °C by a thermostatted water bath. The lipids (DPPC, POPC, DOPC, and Chol) were dissolved in chloroform while DPPG was dissolved in the mixture of chloroform/ethanol 4:1 (v/v) to obtain the solution concentration of about 1 mg/mL. Mixed solutions were prepared from the respective stock solutions. The spreading solutions were dropped onto the water subphase with the Hamilton microsyringe and left for 10 min for solvent evaporation. Then the film was symmetrically compressed at the rate of 4.6 Å^2/molecule·min. All experiments were repeated three times, showing satisfactory reproducibility.

From the slope of the π–A isotherms the compression modulus C_s^{-1} was calculated according to the equation:

$$C_s^{-1} = -A\left(\frac{d\pi}{dA}\right) \tag{1}$$

where A is the area per molecule at the given surface pressure π.[25,26]

Then the excess Gibbs energy of mixing ΔG_{exc} for the binary systems was determined as follows:[27,28]

$$\Delta G_{exc} = \int_0^{\pi} [A_{1,2} - (x_1A_1 + x_2A_2)]\, d\pi] \tag{2}$$

where $A_{1,2}$ is the mean molecular area in the mixed monolayer at a given surface pressure, A_1 and A_2 are the respective molecular areas in the single-component monolayer of components 1 and 2 at the same surface pressure, x_1 and x_2 are the respective mole fractions of the components in the mixed monolayer.

Because the surface pressure of 35 mN/m mimics the lateral pressure in biological membranes,[29] the analysis of the obtained results was conducted at this pressure.

Transfer of Monolayer onto the Mica Surface. Assuming that the molecular organization in the monolayers at the air/water interface does not change when deposited onto the solid support,[30] the investigated films were transferred onto the hydrophilic mica plates at the surface pressure of 35 mN/m. The deposition of monolayers was conducted by drawing vertically the mica plate from the subphase in the Langmuir–Blodgett trough at the rate of 5 mm/min through the monolayer spread at the air–water interface. The constant surface pressure of the lipid monolayer, the rate of deposition, and the temperature were kept constant. For each deposition the transfer ratio was 1.00 ± 0.01. Thus obtained samples were put into the vacuum apparatus under the pressure of 117 mbar for about 20 h at room temperature.

Surface Free Energy Calculation. The advancing contact angles of water (Milli-Q Plus system), formamide (98%, Aldrich), and diiodomethane (99%, Aldrich) were measured on the above-described lipid monolayers, using a GBX contact angle meter (France) equipped with an automatic drop deposition system, with the camera and computer software for the contact angle measurement calculated from the shape of sessile droplet. The droplets of 3-μL volume were used to measure the advancing contact angles. The measurements were conducted in a closed chamber at controlled humidity (about 15% and maintained by a constant flow rate of nitrogen). The measurements of advancing contact angles were repeated in three separate experiments with the reproducibility within 2–4°.

From the measured advancing contact angles (θ_a) of these three liquids (two bipolar and one nonpolar), the changes in the total surface free energy of the lipid monolayers, γ_S^{tot}, were calculated on the basis of the van Oss et al. (LWAB) approach.[22−24] These authors expressed the surface free energy as a sum of apolar Lifshitz–van der Waals, γ_S^{LW}, and polar acid–base, γ_S^{AB}, components:

$$\gamma_S^{tot} = \gamma_S^{LW} + \gamma_S^{AB} \tag{3}$$

The acid–base component is a geometric mean of two parameters:

$$\gamma_S^{AB} = 2\sqrt{\gamma_S^- \gamma_S^+} \tag{4}$$

where γ_S^- is the electron-donor (Lewis base) parameter and γ_S^+ is the electron-acceptor (Lewis acid) parameter.

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On the other hand, the solid/liquid interfacial free energy, γ_{SL}, can be expressed as:

$$\gamma_{SL} = \gamma_S + \gamma_L - W_A^a \quad (5)$$

where γ_S stands for the surface free energy of solid, γ_L is the surface free energy of liquid (in the case of pure liquid, it is numerically equal to the liquid surface tension), W_A^a denotes the work of adhesion of a liquid to a solid surface, and it reads:

$$W_A^a = 2\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + 2\sqrt{\gamma_S^+\gamma_L^-} + 2\sqrt{\gamma_S^-\gamma_L^+} \quad (6)$$

Combining eqs 5 and 6 with Young's equation:

$$\gamma_S = \gamma_{SL} + \gamma_L \cos\theta_a \quad (7)$$

the work of adhesion is expressed as follows:

$$W_A^a = \gamma_L(1 + \cos\theta_a) \quad (8)$$

or

$$W_A^a = \gamma_L(1 + \cos\theta_a) = 2\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + 2\sqrt{\gamma_S^+\gamma_L^-} + 2\sqrt{\gamma_S^-\gamma_L^+} \quad (9)$$

where the subscripts S = solid and L = liquid.

The authors of this approach assumed that for water $\gamma_L^- = \gamma_L^+ = 25.5$ mJ/m^2 and $\gamma_L^{LW} = 21.8$ mJ/m^2, and basing on it the components of other probe liquids were calculated. The surface tension (γ_L) and its components (γ_L^{LW},γ_L^-,γ_L^+)[31] of all the probe liquids: water (W), formamide (F) and diiodomethane (DM) are listed in Table 2.

Table 2. Surface Tension and Its Components of the Probe Liquids in mN/m[31]

probe liquid	γ_L	γ_L^{LW}	γ_L^+	γ_L^-	γ_L^{AB}
water, H_2O	72.8	21.8	25.5	25.5	51.0
formamide, $HCONH_2$	58.0	39.0	2.28	39.6	19.0
diiodomethane, CH_2I_2	50.8	50.8	0	0	0

Moreover, there is an axiom here that, irrespective of the kind of probe liquid used, strengths of the interactions coming from the solid side do not change, which is debatable. Therefore, it is obvious that the thus calculated values of solid surface free energy and its components are relative ones, the more that equality of γ^+ and γ^- for water was assumed.

Knowing the surface tension components of probe liquids, γ_L^{LW}, γ_L^+, γ_L^- (Table 2) the three equations of the same type as eq 9 have to be solved simultaneously to calculate the unknown components of the solid surface free energy, γ_S^{LW}, γ_S^+, γ_S^- and then its total value, γ_S^{tot}. To explain this procedure, the set of three equations (eqs 10a,b,c) is written below in which the values of the surface tension and its components (Table 2) for water (eq 10a), formamide (eq 10b), and diiodomethane (eq 10c) are introduced.

$$\begin{cases} 72.8(1 + \cos\theta_a^W) = 2\sqrt{21.8\gamma_S^{LW}} + 2\sqrt{25.5\gamma_S^+} + 2\sqrt{25.5\gamma_S^-} & (a) \\ 58.0(1 + \cos\theta_a^F) = 2\sqrt{39.0\gamma_S^{LW}} + 2\sqrt{39.6\gamma_S^+} + 2\sqrt{2.28\gamma_S^-} & (b) \\ 50.8(1 + \cos\theta_a^{DM}) = 2\sqrt{50.8\gamma_S^{LW}} & (c) \end{cases} \quad (10a,b,c)$$

where the superscripts denote: W: water, F: formamide, and DM: diiodomethane.

As results from Table 2 show, diiodomethane interacts by dispersion forces only because the electron-acceptor and electron-donor parameters of the surface tension are equal to zero ($\gamma_L^+ = \gamma_L^- = 0$). Thus, eq 10c is reduced, and γ_S^{LW} can be easily calculated from measured contact angles of diiodomethane (θ_a^{DM}) solely. Then, solving the set of two equations (i.e. eqs 10a and 10b) γ_S^+ and γ_S^- can be obtained. Next, substituting γ_S^+ and γ_S^- values in eq 4, the polar component γ_S^{AB} is determined which together with γ_S^{LW} allows for calculation of γ_S^{tot} from eq 3.

3. RESULTS AND DISCUSSION

Condensing Effect of Cholesterol. Figure 1 illustrates the experimental results of average molecular area per molecule as a function of cholesterol concentration. The average area values were taken directly from the pressure−area measurements at the fixed pressure of 35 mN/m for a series of phospholipid/cholesterol compositions.

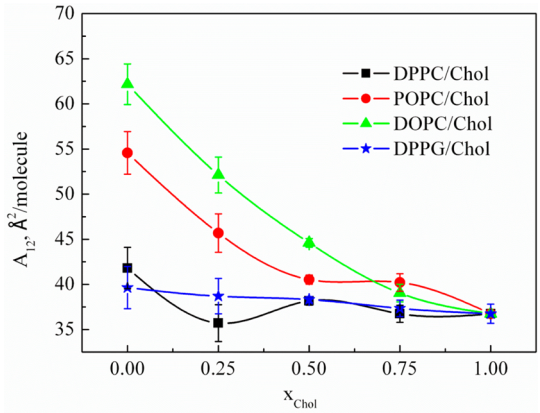

Figure 1. Mean area per molecule (A_{12}) in the film vs cholesterol molar fraction (x_{Chol}) in the mixed monolayers: DPPC/Chol, POPC/Chol, DOPC/Chol, and DPPG/Chol at the surface pressure of 35 mN/m.

Figure 1 convincingly illustrates that the incorporation of cholesterol into the DOPC or the POPC layer leads to reduction of the mean molecular area. In the case of DPPC the cholesterol effect is less distinct, but as a result of area contraction some deviations from linearity (or inflections) in the plots of average molecular area vs composition are observed (Figure 1). These deviations from ideal behavior are due to the existence of lateral interactions between molecules in mixed films. On the contrary, lack of deviations from the area−composition linearity indicates nearly ideal behavior of DPPG/Chol mixtures. The average molecular areas were found to be similar for the systems containing saturated phospholipids (DPPC and DPPG) and Chol. However, they are smaller than those determined for the binary monolayers with unsaturated phospholipids (POPC and DOPC) and Chol. This proves that occurrence of the condensation effect depends on the physical state of lipid monolayers which is affected by the saturation of hydrocarbon chains. However, the magnitude of the area-condensing effect is used only as a rough indicator of the relative strength of interaction between cholesterol and phospholipids. In order to quantify the deviations from ideal behavior, the excess Gibbs energy of mixing (ΔG_{exc}) was

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calculated according to eq 2.[27,28] The dependence of ΔG_{exc} on the layer compositions, determined at 35 mN/m for the all investigated systems, is presented in Figure 2. The ΔG_{exc} values

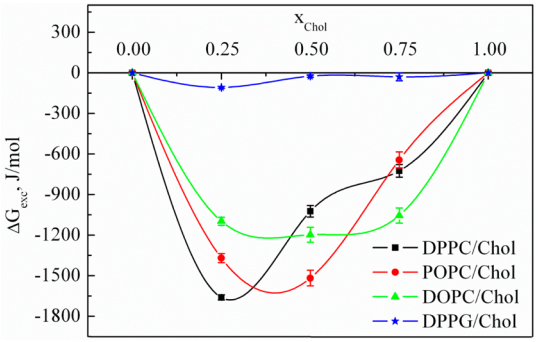

Figure 2. Excess free energy of mixing (ΔG_{exc}) vs cholesterol molar fraction (x_{Chol}) in the mixed monolayers: DPPC/Chol, POPC/Chol, DOPC/Chol, and DPPG/Chol at the surface pressure of 35 mN/m.

allow quantitative evaluation of the magnitude of the interactions between phospholipids and cholesterol, and thermodynamic assessment of the stability of binary monolayers formed.

The run of curves of ΔG_{exc} vs monolayer composition confirms the nonideal phospholipid/cholesterol film behavior. The negative values of ΔG_{exc} in the whole range of monolayer compositions indicate that the insertion of Chol into the phospholipid monolayer causes formation of stable films at their favorable arrangement. However, strong dependence of Gibbs energy on the kind of phospholipid used in the mixture with cholesterol can be seen.

The most negative ΔG_{exc} value (the strongest attractive interaction) was obtained for the DPPC/Chol system at x_{Chol} = 0.25, which makes this mixed monolayer the most stable. For DPPG/Chol a slight minimum in ΔG_{exc}also appears at x_{Chol} = 0.25, but its value is small. Because the saturation of the DPPC and DPPG acyl chains is the same, therefore the type of polar groups must be responsible for the different interactions observed between these phospholipids and cholesterol in the mixed monolayers. Because of drastically smaller negative ΔG_{exc} of DPPG/Chol film in comparison to that of DPPC/Chol, it may be concluded that miscibility of cholesterol in the DPPG monolayer is lower than in the corresponding DPPC layer. On the other hand, for the mixtures of cholesterol with unsaturated POPC and DOPC, the minimum ΔG_{exc} occurs at x_{Chol} = 0.5, but the values are less negative than that of DPPC. This suggests that attractive interactions of unsaturated phospholipids and cholesterol are weaker than those of DPPC and cholesterol. These observations are consistent with literature data. Silvius reviewed findings obtained by various physical techniques relating to the preferential interaction of cholesterol with different classes or molecular species of lipids.[2] In general, calorimetric studies, monolayer measurements, and direct assays of cholesterol partitioning between different bilayer environments show greater cholesterol affinity for fully saturated phospholipids which decreases with the increasing unsaturation of the lipid acyl chains. For instance, differential scanning calorimetry (DSC) studies on the effect of cholesterol on the phase transitions in binary phospholipid/cholesterol mixtures revealed that cholesterol strongly broadens the sharp phase transitions of saturated phospholipids contrary to polyunsaturated chains, indicating a preferential interaction with the saturated species.[32,33] Brzustowicz et al. reported that the solubility of cholesterol in unsaturated phosphatidylcholines is substantially lower than that in saturated or monounsaturated species due to weak interactions with cholesterol.[34] Lange et al. showed that cholesterol partitions with greater affinity into vesicles prepared from a saturated phosphatidylcholine than into vesicles prepared from an unsaturated phosphatidylcholine.[35] Scherfeld et al., taking the lipid diffusion coefficient as a measure of the strength of the phospholipid/cholesterol interaction, provided evidence that cholesterol causes the "largest fluidizing" effect in the domain with lowest mobility, implying that it interacts preferentially with DPPC to DOPC.[36]

Moreover, cholesterol's preference for specific fatty acid chains was investigated in a molecular dynamics computer simulation. The simulations conducted by Pitman prove that cholesterol prefers to be solvated by saturated acyl chains than by unsaturated ones that arise from differences in packing of the hydrophobic core.[37] The affinity for saturated chains provokes formation of compositional bias. Similarly, the simulations by de Joannis et al. demonstrate that cholesterol's higher affinity for DPPC than for DOPC depends on its alignment with the bilayer normal.[38] The presence of kinks induced by cis double bonds in DOPC tails makes the molecule less suited to packing among aligned cholesterols, relative to DPPC. Placing cholesterol next to the disordered unsaturated oleoyl chains imposes the restrictions in their conformations, resulting in an entropic penalty that is relieved when cholesterol is placed next to the ordered, saturated palmitoyl chains.[39]

This favorable interaction may provoke formation of segregated cholesterol-depleted and cholesterol-enriched domains,[3] thus supporting the lipid raft hypothesis. On this basis, it may be assumed that the stable complexes of saturated phospholipids and cholesterol of the defined stoichiometry are formed at the air/water interface.

Some authors claim that the plots of area vs composition, showing a large change in the slope, can be attributed to phospholipid/cholesterol complex formation, and hence, the average molecular area of the binary complex can be estimated.[7] In the simulations of average area vs composition plots conducted by McConnell and Radhakrishnan,[7] which are in line with the experiments carried out by other investigators,[40−42] some distinct behavior of the condensed complexes was found. For the weak complexes, no sharp breakpoints in the plots were visible, while for the stronger ones a distinct break was observed at the stoichiometric composition. The above results shed a light on the investigations presented here. The obtained plot inflections (Figure 1) can be attributed to the strong DPPC/Chol and POPC/Chol complex formation at x_{Chol} = 0.25 and 0.5, respectively. The largest negative values of the excess Gibbs energy (Figure 2), indicating stronger attraction between heteromolecules observed at analogous composition of monolayers, seem to confirm this hypothesis. Similar inflections are also seen at the lower surface pressures (not shown here). In the case of DPPG/Chol and DOPC/Chol, although no clear inflections in the plot of average molecular area are seen (Figure 1), the excess free energy plot shows a minimum at x_{Chol} = 0.25 and x_{Chol} = 0.5, respectively (Figure 2). This suggests that a weak complex can form, which however, cannot be observed on the average molecular area plots. This is in agreement with the studies of McConnell and Radhakrishnan which showed that the equivalence point of the complexes formation varies between 25% and 50% of

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cholesterol content.[7] Further increase of cholesterol content results in a phase separation or simply mixing of the cholesterol with the complexes, and then the average area changes only slightly.

However, one can say that the presence of phospholipid/cholesterol complexes can be deduced from qualitative and quantitative analysis of the interactions between molecules. Namely, if in the mixed monolayer the area contraction takes place and if additionally it is confirmed by the negative value of excess Gibbs energy, the values of these two parameters indicate stoichiometry of the binary complexes.

Compression Modulus. The condensing effect of cholesterol on phospholipid monolayers revealed in the contraction of area (Figure 1) can be deduced from various factors such as molecular packing, ordering of chains, and tilting of polar heads. To determine the state of investigated films and to obtain information on the molecular ordering of molecules in the monolayer, the compression modulus was calculated according to eq 1. Figure 3 shows the compression modulus values determined at 35 mN/m vs composition.

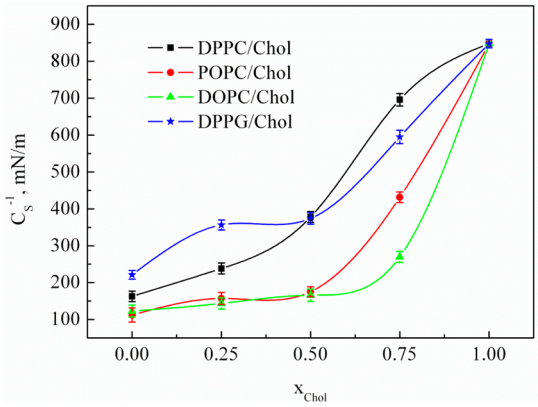

Figure 3. Compression modulus (C_S^{-1}) values vs cholesterol molar fraction (x_{Chol}) for the mixed monolayers: DPPC/Chol, POPC/Chol, DOPC/Chol, and DPPG/Chol at the surface pressure of 35 mN/m.

From C_S^{-1} values it is found that at 35 mN/m the unsaturated monolayers of POPC or DOPC exist as liquid-expanded monolayers which are less packed and less ordered than liquid-condensed saturated monolayers of DPPC and DPPG. In agreement with the results reported by Wydro et al.,[43] at a lower cholesterol concentration (x_{Chol} = 0.25−0.5) the values of compression modulus for DOPC/Chol and POPC/Chol mixtures are convergent and small. On the other hand, for DPPC/Chol and DPPG/Chol an increase of C_S^{-1} is observed, which at higher cholesterol content is also stronger than for the mixtures with unsaturated phospholipids. It is likely that a relatively small polar headgroup of the DPPG molecule in comparison to that of DPPC allows the most compact lipid assembling due to a reduced steric hindrance. The chains of saturated phospholipids are mostly in trans conformation, and the molecules are likely to be closely packed. On the other hand, the presence of double bonds being in the cis configuration in POPC and DOPC molecules induces a kink in the hydrocarbon chain and hampers a very close assembling of the lipids. Hence, one may conclude that the mixtures of cholesterol with saturated phospholipids (DPPC, DPPG) form more condensed and chain-ordered films than those containing unsaturated phospholipids (POPC, DOPC). This is also reflected in smaller area A_{12} values in the monolayers containing saturated phospholipids (Figure 1). Closer packing and chain-ordering in the films may facilitate binary complex formation at defined stoichiometry.

Surface Free Energy Determined From the LWAB Model. The monolayers deposited on mica were investigated with respect to their surface free energy. The contact angle measurements of probe liquids with their known surface tension contributions and appropriate theoretical approaches are needed to determine the surface free energy of solids. In this report van Oss et al. approach (LWAB) of the surface free energy evaluation was applied (eq 9).[22−24] The values of total surface free energy of one-component and binary monolayers vs the molar fraction of cholesterol are shown in Figure 4. As

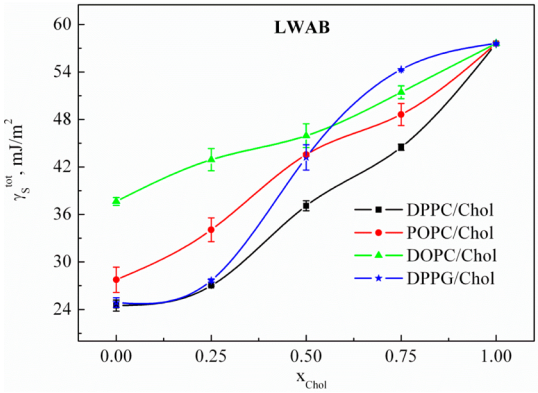

Figure 4. Total surface free energy, γ_S^{tot}, calculated from the LWAB approach vs cholesterol molar fraction (x_{Chol}) for the mixed monolayers: DPPC/Chol, POPC/Chol, DOPC/Chol, and DPPG/Chol deposited on mica at 35 mN/m.

can be seen, the surface free energy changes, depending on the type of phospholipid and the ratio of cholesterol to phospholipid. The presence of an increasing amount of cholesterol in the phospholipid monolayer causes a gradual increase of the energy. The surface free energy of monolayers composed of saturated DPPC is always lower than those built up of unsaturated POPC and DOPC. These zwitterionic phospholipids have the same headgroup (phosphocholine) but different degrees of saturation of the acyl chain. The presence of one (POPC) or two (DOPC) unsaturated bond(s) in the phospholipid molecule markedly increases the surface free energy of lipid films.

To explain such energy changes, the ordering of molecules in the films should be considered. By analyzing the compression modulus values for the investigated systems (Figure 3), it was found that DPPC and DPPG exist as a liquid-condensed phase, whereas POPC and DOPC occur as a liquid-expanded phase. With the increase in cholesterol amount the molecules become more densely packed, and a stronger ordering effect on saturated DPPC and DPPG as compared to that of unsaturated POPC and DOPC phospholipids at all monolayer compositions is observed.

It should be also stressed that the surface pressure of 35 mN/m in a lipid monolayer corresponds to lateral pressure in a lipid bilayer at atmospheric pressure and if external stress is absent.[29] Therefore, assuming that a closely packed state of the monolayers is preserved after their deposition onto the mica support, the temperature of main gel−fluid transition of the transferred monolayers is the same as that of the phospholipid

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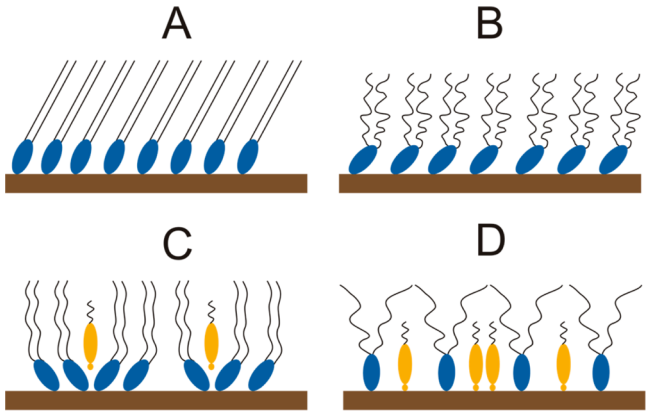
bilayers in water. Accordingly, since at 20 °C and 35 mN/m, the phase transition temperatures are 41 °C for DPPC, 41.5 °C for DPPG, −3 °C for POPC, and −18 °C for DOPC,[3] then DPPC and DPPG form an ordered gel phase, but POPC and DOPC monolayers occur in a disordered liquid-crystalline (fluid) phase. Models illustrating the different physical states of monolayers are depicted in Scheme 1.

Scheme 1. Models Illustrating Different Physical States of Monolayers: Solid-Ordered (Gel) Phase (A), Liquid-Disordered (Fluid) Phase (B) of Pure Phospholipid Film, and Liquid-Ordered (Fluid) Phase of Phospholipid/Cholesterol Film at Low Cholesterol Content (C) and at High Cholesterol Content (D)



However, the increasing amount of cholesterol in the phospholipid monolayer progressively converts both the gel state of DPPC and DPPG films (Scheme 1A) and the liquid-crystalline state of POPC and DOPC monolayers (Scheme 1B) to the liquid-ordered state (or liquid-crystalline-ordered state) which shares the characteristics of both gel and fluid phases[44] (Scheme 1C and D). In other words, the presence of cholesterol in a solid-ordered phase reduces ordering of the lipid chains, but in the case of a liquid-crystalline-disordered phase an increase in the ordering of molecules takes place. The probe liquids used for the contact angle measurements more easily penetrate the films composed of unsaturated phospholipids whose packing is looser, and therefore the molecules can interact with liquids by both hydrogen bonds and polar forces. Therefore, the surface free energy of POPC/Chol and DOPC/Chol films is always higher as compared to DPPC/Chol monolayers.

The surface free energy increase may be caused not only by the changes in tight packing and ordering of the molecules in monolayer but also by changes in tilting of the molecules, which changes the monolayer permeability for small molecules of the liquids. Ivankin et al., using X-ray reflectivity (XR) and grazing incidence X-ray diffraction (GIXD), demonstrate the dependence of the cholesterol position with respect to the DPPC molecules on the layer composition.[17] Namely, in the range of x_{Chol} = 0.13−0.46 cholesterol is located within the hydrophobic region of DPPC molecules, whereas their phosphocholine groups are tilted from the normal of the surface (Scheme 1C). For the mixtures with x_{Chol} = 0.7 and 0.85 cholesterol molecules descend into the headgroup region of DPPC, thus provoking the tilt of DPPC acyl chains (Scheme 1D). Similarly, Wydro et al. claim that an increase of the cholesterol fraction changes the monolayer condensation as a result of the change in tilting of the phosphocholine group. The tilt of polar groups in more fluid monolayers (DOPC, POPC) is larger than in the gel film. Therefore, the increase of cholesterol concentration more strongly influences the tilt of the headgroup of unsaturated phospholipids.[43] The tilt of headgroups or chains in the multicomponent monolayers may perturb the local packing of the molecules being the driving force of the cholesterol precipitation or patched film formation when an appropriate concentration of cholesterol is reached.

Moreover, mismatch in length and cross section between the cholesterol molecule and the phospholipid molecule (Scheme 1) is believed to create some cavities in the monolayer structure, which make the liquid penetration easier. The length and cross section of a cholesterol molecule are approximately 1.7 nm[45] and 0.37 nm^2, respectively, while those of a phospholipid molecule are about 3 nm and 0.42−0.62 nm^2, respectively, depending on the kind of phospholipid.

4. CONCLUSIONS

From the Langmuir and Langmuir−Blodgett monolayers studies it is found that some correlation between the magnitude of phospholipid/cholesterol interactions and the total surface free energy of the investigated monolayers occurs. For all phospholipid/cholesterol systems the gradual increase in the surface free energy, as determined from the LWAB approach, with the increasing amount of cholesterol is observed. However, this increase is always larger for unsaturated than for saturated phospholipids. These changes are determined by the kind of phospholipid, organization, packing, and ordering of the molecules in the monolayers. It is believed that these results are interesting for better understanding of the organization of lipid rafts in the mammalian cell membranes and their interactions with the surrounding environment.

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Notes

The authors declare no competing financial interest.

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