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Interfacial tension of phosphatidylcholine–cholesterol system in monolayers at the air/water interface

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Abstract

Interfacial tension of an egg lecithin–cholesterol system was measured across the whole concentration range. Surface pressure–area isotherm measurements were carried out in a Langmuir trough at the air/water interface at room temperature (22 °C). The interfacial tension of the air/water interface was divided into contributions of components. The interfacial tension of a 1:1 complex between phosphatidylcholine and cholesterol was calculated. Its value equals 18 mN/m. The difference between the stability constant of 1:1 complex in the bilayer and the monolayer at the air/water interface is discussed.

Keywords: Egg lecithin; Cholesterol; Interfacial tension; Monolayer; Equilibrium

1. Introduction

Three typical models of biological membranes are planar lipid bilayers, vesicles (liposomes) and monolayers [1]. According to Singer–Nicholson's model of a cell membrane [2], a lipid bilayer resembles a biomembrane closer than a monolayer.

Most lipid monolayer studies concentrate on surface potential or surface pressure measurements, spectroscopy and microscopic visualisation of lateral domains [3]. Also, several techniques based on the Langmuir–Blodgett method are widely used for such investigations [4]. In spite of a wide variety of experimental methods to study lipid monolayers, some long-lasting problems remain. One of them is the complex formation between phospholipids and cholesterol in a monolayer at the air/water interface or in a bilayer [5].

The phosphatidylcholine–cholesterol system is widely studied because of its existence in biological membranes and interesting features of condensation effect of cholesterol [6,7]. There are several various explanations for this effect on the membranes. One is the complex formation in the monolayer at the air/water interface. Assumptions of the existence of a complex at different stoichiometries in a lipid monolayer have been confirmed by several experimental techniques [8,9]. However, there is no agreement concerning whether the complexes exist and what stoichiometry they have. The 1:1, 1:2, 2:1, 1:3, 3:1 complexes are mostly

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claimed to be in monolayers or bilayers [10]. However, some models concerning the existence of phospholipid–cholesterol complexes in the bilayer suggest that there is no favourable interaction between the mentioned lipids. Huang and Feigenson suggest that phospholipid groups act as 'umbrellas', shielding the non-polar part of cholesterol from water in a lipid bilayer (so-called 'umbrella model') [11].

The monolayer–bilayer correspondence problem has been widely studied, both experimentally and theoretically [11–14]. Information obtained from simulations of bilayers suggests that long-range forces are probably responsible for disagreements in simulation and experimental data. The lipid bilayers are more sensitive to changes in surface area than monolayers.

A model of a bilayer composed of two monolayers connected back-to-back, with negligible interactions between the two leaflets, was studied by Nagle [15,16]. He indicated that, in the case of monolayers at an air/water interface, one obtains an additional interface (in comparison to the analogous bilayer) of air/hydrophobic parts of molecules. However, assuming that the bilayer is a system of two monolayers, many researchers concentrate on monolayers in order to apply data obtained from experiments on monolayers to bilayers.

In the opinion of Gruen and Wolfe, a monolayer at an *n*-alkane/water interface is a closer analogy of half of a lipid bilayer than a monolayer at the air/water interface, at the same area per molecule [17]. The roughness of the air/lipid chain interface is the reason why the interfacial tension of hydrocarbon layer of monolayer/air is dependent on the lipid surface area [17]. However, due to the equilibrium state where most of experiments are carried out, this roughness is assumed not to influence the latter interface strongly [13]. It may be quite different in the case of the lipid bilayer. In a bilayer composed of two compounds with different molecular lengths or different structures, the area of hydrophobic surface is enlarged. The roughness in a bilayer can influence some of the properties of the bilayer, in contrast to a monolayer. It can be interpreted by an assumption of additional complex formation between two hydrophobic layers of bilayer.

Under specific surface pressure (similar in numbers to the interfacial tension between oil and water), the phase transition temperature for monolayers and bilayers is the same. Monolayers at the water/air interface can have the same thermodynamic properties as a leaflet of vesicle under specific conditions [18]. The molecular packing and lateral pressures of the equilibrium monolayer and equivalent vesicles are virtually the same [16].

In our earlier paper, we presented equations for the calculation of the stability constant of a 1:1 phosphatidylcholine–cholesterol complex and area per such molecule in the monolayer at the air/water interface [19]. Our considerations were based on the surface pressure–area curves. These studies are now extended by interfacial tension measurements of mixed lipid monolayers at the air/water interface. The surface tension of the air/hydrophobic interface of a 1:1 L–Ch complex is calculated. We verify the usefulness of equations applied for bilayer in monolayer system at the air/water interface.

2. Theory

In a lipid bilayer, one can distinguish three surfaces: two of hydrophilic parts of molecules/aqueous electrolyte and one between the hydrophobic parts of two layers of the bilayer. The latter interface is assumed to have very small interfacial tension values, almost equal to zero, so the two hydrophilic/electrolyte interfaces contribute practically exclusively to the interfacial tension value of bilayer. In the case of the monolayer, the situation is quite different. There are two interfaces: the first one is the hydrophobic region of molecules/air interface and the second one is the hydrophilic parts of molecules/aqueous electrolyte interface.

The equations derived for the calculation of interfacial tension values were previously used for lipid bilayers [20]. Nevertheless, whether it is a bilayer or a monolayer, each two-compound system can be described by the equations system presented below:

$$\gamma_1 c_{S1} A_1 + \gamma_2 c_{S2} A_2 = \gamma \quad (1)$$

$$\frac{c_1}{c_1 + c_2} = x_1; \quad \frac{c_2}{c_1 + c_2} = x_2;$$

- where: γ_i is the interfacial tension of compound i (at the air/water interface for monolayers or at the hydrophobic layer/aqueous electrolyte interface for bilayers) [mN/m];
- c_{Si} is the surface concentration of compound i in the monolayer [mol/m^2];
- c_i is the total surface concentration of compound i in a monolayer or a half of bilayer [mol/m^2];
- A_i is the area per molecule of compound i [m^2/molec];
- x_i is the molar fraction of compound i; and
- Subscripts 1 and 2 denote egg lecithin and cholesterol, respectively.

One can modify the above equations system to the linear form:

$$(\gamma - \gamma_1) x_1 = \frac{A_2}{A_1} (\gamma_2 - \gamma) x_2 \quad (2)$$

Let us assume that there is the equilibrium in the system, regardless if it is a monolayer or a bilayer. As a result of this equilibrium a chemical complex with the stoichiometry of 1:1 is formed [9,10]. Let us denote it by subscript '3'.

$$L_1 + L_2 \rightleftarrows L_3$$

Assuming that the interfacial tension γ is the sum of the contributions of components, Eq. (1) is modified. Then:

$$\gamma_1 c_{S1} A_1 + \gamma_2 c_{S2} A_2 + \gamma_3 c_{S3} A_3 = \gamma \quad (3)$$

$$K = \frac{c_{S3}}{c_{S1} c_{S2}}$$

$$x_1 = \frac{c_{S1} + c_{S3}}{c_{S1} + c_{S2} + 2c_{S3}}$$

$$c_1 = c_{S1} + c_{S3}$$

$$c_2 = c_{S2} + c_{S3}$$

where K denotes the stability constant of complex formed. Two substances can form complexes at different stoichiometries. However, due to the fact that the first stability constant in complexes, as the essential one, is usually the biggest and should be taken into consideration [21], we assume that a 1:1 complex is formed in that system. Eq. (3) can be presented in different form, more suitable for calculations:

$$\gamma - \gamma_1 c_{S1} A_1 - \gamma_2 c_{S2} A_2 = \gamma_3 c_{S3} A_3 \quad (4)$$

On this basis, knowing the A_i values from, e.g. surface pressure–area isotherms [11], one may obtain the equation where γ_3 is the slope of the line described by Eq. (4).

$$\gamma = \frac{A_1 \gamma_1 + x_2 (A_3 \gamma_3 - 2A_1 \gamma_1)}{A_1 + x_2 (A_3 - 2A_1)} \quad (5)$$

$$\gamma = \frac{A_3 \gamma_3 - A_2 \gamma_2 + x_2 (2A_2 \gamma_2 - A_3 \gamma_3)}{A_3 - A_2 + x_2 (2A_2 - A_3)} \quad (6)$$

Eqs. (5) and (6) describe the dependency of interfacial tension function for $x_2 < 0.5$ and big $x_2 > 0.5$ values, respectively, providing the stability constant K is big enough. It is worth emphasising the fact that at a molar fraction of cholesterol $x_2 = 0.5$, the value of interfacial tension is equal to that of the complex ($\gamma = \gamma_3$).

In some cases, other equations can be useful, e.g. slopes, for $x_2 \to 0$:

$$\frac{d\gamma}{dx_2} = \frac{K \frac{A_3}{A_1} (\gamma_3 - \gamma_1) - A_2 (\gamma_1 - \gamma_2)}{K + A_1} \quad (7)$$

or for $x_2 \to 1$:

$$\frac{d\gamma}{dx_2} = - \frac{K \frac{A_3}{A_2} (\gamma_3 - \gamma_2) - A_1 (\gamma_1 - \gamma_2)}{K + A_2} \quad (8)$$

Eqs. (7) and (8) can be used for verification of the values of the slopes of experimental curves obtained from the interfacial tension values vs. composition. Good agreement between slope of experimental data and that calculated from Eqs. (7) and (8) means that the system is well described by these equations.

3. Materials and experimental details

The egg lecithin (3-sn-phosphatidylcholine of hen eggs yolk) ~99% (TLC) from Fluka was

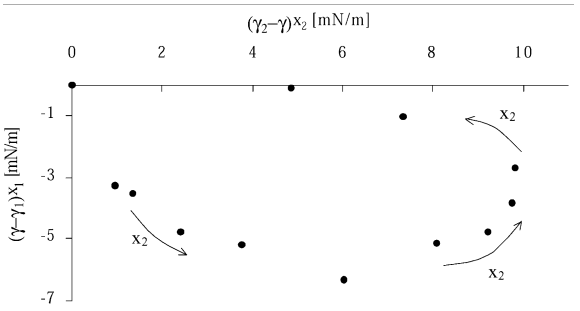

Fig. 1. The dependence of $(\gamma-\gamma_1)\cdot x_1$ vs. $(\gamma_2-\gamma)\cdot x_2$. The arrows denote the direction of increasing x_2 values.

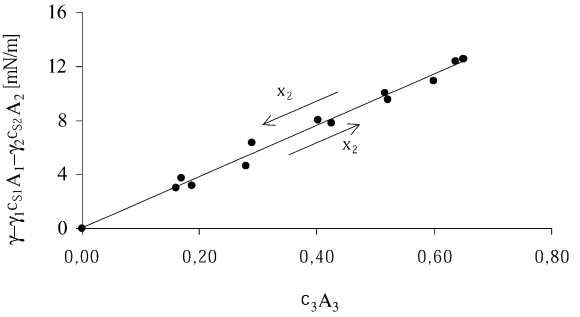

Fig. 2. The dependence of $(\gamma-\gamma_1 c_{S1}A_1-\gamma c_{S2}A_2)$ as a function of $c_{S3}A_3$. The arrows denote the direction of increasing x_2 values.

used without further purification. Cholesterol from POCh Gliwice (Poland) was crystallised twice from methanol. 1-Chloropropane from Fluka was used as a spreading solvent [19]. The water used was triply distilled.

The measuring procedure has been described elsewhere [19]. Surface tension measurements were carried out in Langmuir trough equipped with a 9000 Nima tensiometer at the water/air interface at 22 °C. The mixtures of lipids dissolved in 1-chloropropane, at the required composition, were put on the surface of subphase with a Hamilton syringe. After evaporation of the solvent (10 min), the compression of the monolayer was performed. The rate of compression was 0.1 cm/s.

4. Results and discussion

From a thermodynamic point of view, we can imagine the bilayer as a system which is made up from two nearly independent monolayers with very small attractive interaction between the monolayers [22,23]. As a result, a hydrocarbon/air interface is created in the monolayer case, but not in the bilayer case [15,16], as mentioned above. Jähning proposed that the difference between a monolayer at the water/air interface and one leaflet of vesicles is the excess of the interfacial tension of hydrocarbon layer/air interface [12].

The determination of surface tension of monolayers is relatively straightforward. In case of bilayers, an accurate estimation of direct surface tension is very difficult. On the basis of the Laplace–Young equation, the surface tension values can be determined for lipid bilayers providing knowledge of the pressure difference across the lipid membrane and the radius of curvature [20].

In the Langmuir approach [24], an air/hydrophobic layer and polar layer/aqueous subphase interfacial tensions make up the monolayer surface tension. The difference between the monolayer interfacial tension obtained experimentally and the interfacial tension of bilayer equals the interfacial tension of hydrophobic layer/air. In a further part of this paper, we will apply the values obtained in the way described above to our calculations. We proceed in a similar way as Jähning, who approximated a hydrophobic layer/air to the interfacial tension of the *n*-alkane/air interface [12].

Fig. 1 presents the dependence $(\gamma-\gamma_1)\cdot x_1$ vs. $(\gamma_2-\gamma)\cdot x_2$ described by Eq. (2). According to Eq. (2) — in the case where complex is not formed — the values of this function should form a straight line. As one can see, this is not the case, which suggests that there is a complex or chemical compound formation in such system. Since Eq. (2) does not describe the system under study sufficiently, we assume, on the basis of literature [9], the existence of a complex between phosphatidylcholine and cholesterol. Consequently, Eq. (3) and the stability constant *K*, describing a third compound formed in this system, broaden the theoretical description. After simple modifications of Eq. (3), one can obtain information of great interest from our point of view, presented by Eq. (4).

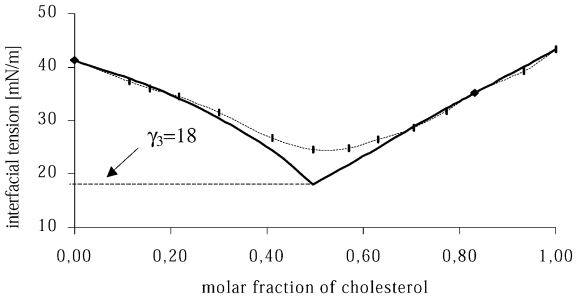

Fig. 3. The interfacial tension of egg lecithin–cholesterol system vs. composition. Points denote experimental values and solid lines the theoretical curves, the spaced line is the theoretical curved calculated on the basis of Eq. (3).

Fig. 2 shows the dependence illustrating the Eq. (4). Because the existence of a 1:1 complex is essential for others, as was written earlier, and based on the experimental data, we assume that a 1:1 complex between phosphatidylcholine and cholesterol is formed. The drawn plot of Eq. (4), provided that a 1:1 phosphatidylcholine–cholesterol complex is formed, shows a straight line with a slope equal to the required value of interfacial tension of complex. In our case, γ_3 is equal to 18 mN/m with the correlation coefficient $R^2 = 0.9363$.

Fig. 3 presents the interfacial tension values of the air/hydrophobic layer of egg lecithin–cholesterol system as a function of composition. The γ_1 value for a pure monolayer of egg lecithin is equal to 41.25 mN/m and γ_2, for a monolayer of pure cholesterol, equals 43.25 mN/m. Points denote experimental values. The dotted line is calculated on the basis of Eq. (3), using the following values obtained from surface pressure–area isotherms: the surface area per egg lecithin molecule equals to 66 Å^2/molec, the surface area per cholesterol molecule equals to 41 Å^2/molec [19], and values of interfacial tension have been already mentioned above.

The agreement between experimental data and theoretical points is good, which verifies the assumption of formation of a 1:1 lecithin–cholesterol complex in the studied system. It could be argued that complexes at different stoichiometry could be formed in this system. Still, the fact remains that we do not distinguish other complexes in the monolayers based on our experimental data. Good agreement between them and the theoretical curve does not indicate the existence of other complexes.

The dependence of interfacial tension of the air/hydrophobic layer on the system composition reveals that the minimum is not situated at a molar fraction of cholesterol of 0.5. This might be due to different values of area per molecule of egg lecithin (66 Å^2/molec) and cholesterol (41 Å^2/molec). In the case where two compounds occupy the same area, the minimum would be exactly at $x_2 = 0.5$.

In Fig. 3, points denoting the experimental data and the theoretical curves (solid lines) are calculated using Eqs. (5) and (6) for small and large x_2 values, respectively. From the comparison of the curve representing Eq. (5) and experimental data in the range of molar fraction between 0 and 0.3, it is easily seen that Eq. (5) describes the studied system well, similarly, as Eq. (6) does for the range of x_2 between 0.75 and 1. On this basis, we can conclude that the stability constant is big enough for the complexes in these two regions to be formed.

The difference between experimental points and theoretical curve in the range 0.35–0.7 suggests that the influence of stability constant on complex formation is strong in that region. Provided there is a large value of K, we would get two curves which intersect at $x_2 = 0.5$ (Fig. 3). Then, there are the formed complex and the excess of egg lecithin for the molar fraction of cholesterol in a range between 0 and 0.5. Similarly, a complex is formed and the excess of cholesterol remains in the region where x_2 is bigger than 0.5. However, at $x_2 = 0.5$, there is only a 1:1 complex between egg lecithin and cholesterol in the studied system. Then, a comparison of Eqs. (5) and (6) leads to the equality between γ and γ_3. At a molar fraction of 0.5, the value of the interfacial tension on the y-axis shows the γ_3 value for the complex. It is equal to 18.05 mN/m, the same value that was obtained as a slope of the line in Fig. 2.

The stability constant of the 1:1 complex between egg lecithin and cholesterol amounts to 2.661×10^7 m^2/mol in a bilayer [20], while in an

analogous monolayer, it is 2.56×10^6 m^2/mol [19]. The larger value of stability constant for bilayer (approx. one order of magnitude) probably results from the roughness between the hydrophobic layers in bilayer [17]. Additional complexes between two leaflets of bilayer can be formed due to the roughness of this interface.

The interfacial tension of the 1:1 complex is equal to 4.423 and 18 mN/m in the L–Ch bilayer and L–Ch monolayer, respectively [20]. A 1:1 complex formation in such a bilayer increases the interfacial tension values strongly, especially for egg lecithin, comparing to the interfacial tension values of pure components ($\gamma_{lec}=1.623$ mN/m) and, on the contrary, a complex with a γ_3 value hardly increases the interfacial tension value of cholesterol ($\gamma_{chol}=4.715$ mN/m). In the case of an analogous monolayer, the formation of a 1:1 complex rapidly changes the interfacial tension values for the system under study. The value of the interfacial tension of the complex obtained for the monolayer is two times smaller than the interfacial tension values of pure egg lecithin or cholesterol.

Knowing the stability constant K and the areas per egg lecithin, cholesterol and complex molecules, one can compare the values calculated from Eqs. (7) and (8) and those obtained on the basis of experimental points. It can be used as a method of verification of the obtained K value or the surface area per a molecule of complex A_3.

5. Conclusions

In a lipid monolayer adsorbed at the air/water interface, one can distinguish the following interfaces: hydrophobic parts of molecules/air and polar parts of molecules/water. As a result, the interfacial tension of the lipid monolayer is composed of the values of interfacial tension of individual interfaces. The interfacial tension of the hydrophobic layer/air interface dominates in the phosphatidylcholine–cholesterol monolayer. We considered the existence of a 1:1 complex between egg lecithin and cholesterol. The equation for calculation of the interfacial tension of the complex in a monolayer or in a bilayer is derived. In the case of a lipid monolayer, in the range of molar fraction of cholesterol between 0.35 and 0.7, one can see the existence of both a complex and lipids not forming the complex. The roughness of the two hydrophobic leaflets of lipid bilayer results in the difference between the stability constant of complex in the egg lecithin–cholesterol monolayer ($K=2.56\times10^6$ m^2/mol) and in the L–Ch bilayer (2.661×10^7 m^2/mol).

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