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ORIGINAL PAPER

The Interfacial Tension of the Lipid Membrane Formed from Lipid–Amino Acid Systems

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Abstract The interfacial tension of lipid membranes composed of phosphatidylcholine (lecithin, PC)–valine (Val), phosphatidylcholine–isoleucine (Ile), phosphatidylcholine–tyrosine (Tyr), and phosphatidylcholine–phenylalanine (Phe) has been studied. The membrane components formed 1:1 complexes. The interfacial tension measurements were used to determine the membrane surface concentration A_3^{-1}, the membrane interfacial tension γ_3, and the stability constant K.

Keywords Interfacial tension · Bilayer membrane · Phosphatidylcholine · Valine · Isoleucine · Tyrosine · Phenylalanine · Complex 1:1

Introduction

Natural cell membranes have been studied by numerous techniques including physicochemical ones. An important property of a cell membrane is its interfacial tension, which determines its rigidity and, as a result, affects its stability. A cell membrane is a very complex system, and it contains various structural components that can influence its interfacial tension. Therefore, it is easier to study the effect of various factors; e.g., amino acid–lipid interaction using artificial phospholipid bilayer model membranes. The properties of the artificial membrane should be well known and generally similar to the properties of the membranes of living cells. Lipid monolayers, lipid bilayers, collodion, cellophane, millipore, ion-exchanger, or other membranes have been used as artificial membranes [1], but the interfacial tension of a cell membrane is best measured by means of a spherical bilayer lipid model membrane.

Amino acids are ubiquitously found in all living cells [2, 3]. Therefore, although they are usually only present in low concentrations in cells, amino acids represent interesting model substances to examination the interaction between amino acids and bilayer lipid membranes. These interactions have been examined used numerous experimental studies [4–6]. MacCallum et al. [7, 8] have calculated the distribution in a lipid bilayer of small molecules mimicking 17 natural amino acids in atomistic detail by molecular dynamics simulation. The results give detailed insight in the molecular basis of the preferred location and orientation of each side chain as well as the preferred charge state for ionizable residues.

To understand complex biological systems it is valuable to analyze simpler model systems. It would be useful, for example, to be able to determine the influence of individual amino acids on the interactions of peptides with a cell membrane. Jacobs and White used a variety of techniques to examine the thermodynamics and binding of a general class of tripeptides, to small phosphatidylcholine vesicles [9]. These experiments revealed peptide induced alterations of the lipid order and modulations of the lipid acyl chain motion. Later work related these thermodynamic parameters with structural information from neutron diffraction experiments [10]. These experiments were used to determine the general location of the peptide in the bilayer, if the peptide inserted in hydrocarbon region or was confined to the water–hydrocarbon interface. Specific information concerning the structure of the lipid–peptide complex

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would be extremely useful in further understanding the role of individual amino acids in peptide–lipid interactions.

It is well known the importance of investigations of interactions between proteins and biological membranes. We decided to give a deeper sight to this problem by the determination of ways of interactions between main structural elements of proteins and membranes: amino acid molecules and phospholipid bilayers correspondingly, using interfacial tension method. By means of interfacial tension method, remarkable progress is being made in the understanding of lipid bilayer behavior influence on the peptide–lipid interaction.

The effects of membrane composition on interfacial tension were previously described in phosphatidylcholine–other lipid [11], phosphatidylcholine–fatty acid, and phosphatidylcholine–amine systems [12]. This work is a continuation of our studies concerning the interaction of lipid bilayers with molecules of increasing complexity using membranes composed of phosphatidylcholine–amino acid system. We describe the dependence of interfacial tension on membrane composition in phosphatidylcholine (PC)–valine (Val), PC–isoleucine (Ile), PC–tyrosine (Tyr), and PC–phenylalanine (Phe) over a possible range of compositions, and present a comparison of the stability constants of the complexes and the surface areas occupied by the membrane components.

Theory

In the cases where the membrane components do not form chemical compounds, their interaction can be described by the following set of equations [13, 14]:

$$\begin{aligned} \gamma_1 m_1 A_1 + \gamma_2 m_2 A_2 &= \gamma \\ \frac{m_1}{m_1 + m_2} &= x_1 \\ x_1 + x_2 &= 1 \end{aligned} \tag{1}$$

where A_1^{-1}, A_2^{-1} (mol m^{-2}) are the surface concentration of components 1 and 2; m_1, m_2 (mol m^{-2}) are the quantities of components 1 and 2 per unit area of the membrane; γ_1, γ_2 (N m^{-1}) are the interfacial tensions of membranes assembled from pure components 1 and 2; γ (N m^{-1}) is the measured interfacial tension of the membrane; and x_1, x_2 are the solution mole fractions of components 1 and 2.

The elimination of m_1 and m_2 yields the linear equation:

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

Membranes may also be assembled from two components capable of forming a complex. The stoichiometry of the complex may vary, but because the first stability constant in these complexes is usually the largest [15], we assumed that the complexes are primarily of 1:1 stoichiometry.

In cases where the membrane components form a 1:1 complex, interactions in the membrane may be described by a previously published set of equations [14].

The equilibrium between the individual components and the complex is represented by:

$$\text{A (Component 1)} + \text{B (Component 2)} \Leftrightarrow \text{AB (Complex)}$$

and the basic equation describing the interaction between components 1 and 2 can be written as [11, 14]:

$$\begin{aligned} &[(\gamma - \gamma_1)B_2x_1 + (\gamma - \gamma_2)B_1x_2][(\gamma_3 - \gamma_1)B_2x_1 \\ &+(\gamma_3 - \gamma_2)B_1x_2 + (\gamma_1 - \gamma_2)(x_1 - x_2)] \\ &= KA_3^{-1}B_1B_2[(\gamma - \gamma_1)(x_2 - x_1) \\ &\quad +(\gamma_3 - \gamma)B_1x_2][(\gamma - \gamma_2)(x_1 - x_2) + (\gamma_3 - \gamma)B_2x_1] \end{aligned} \tag{3}$$

where $B_1 = A_3/A_1$ and $B_2 = A_3/A_2$.

Equation 3 may be simplified by taking into account the high stability constant of the complex. Applying this simplification results in linear behavior for small ($x_2 < x_1$) and large ($x_2 > x_1$) x_2 values [11, 14].

$$(\gamma_1 - \gamma)\frac{x_1 - x_2}{x_2} = -B_1\gamma_3 + B_1\gamma \tag{4}$$

$$(\gamma_2 - \gamma)\frac{x_2 - x_1}{x_1} = -B_2\gamma_3 + B_2\gamma \tag{5}$$

When calculating the stability constant for the complex, Eq. 3 can be simplified to $x_1 = x_2$ [11].

$$\begin{aligned} &K\left(A_1^{-1}\right)^2\left(A_2^{-1}\right)^2\left(A_3^{-1}\right)^{-1}(\gamma - \gamma_3)^2 \\ &= \left[\gamma_2A_1^{-1} + \gamma_1A_2^{-1} - \gamma\left(A_1^{-1} + A_2^{-1}\right)\right]\left(\gamma_2A_1^{-1} + \gamma_1A_2^{-1}\right) \\ &\quad - \left[\gamma_2A_1^{-1} + \gamma_1A_2^{-1} - \gamma\left(A_1^{-1} + A_2^{-1}\right)\right]\left(A_1^{-1} + A_2^{-1}\right)\gamma_3 \end{aligned} \tag{6}$$

The parameters describing the complex may be used to calculate theoretical points using the equation presented below (agreement between the theoretical and experimental values implies that the system is well described by the above equations):

$$\begin{aligned} &KA_1^{-1}A_2^{-1}(a_1 + a_2)(a_3 - a_1)\gamma^2 \\ &+ \left[KA_1^{-1}A_2^{-1}(\gamma_1a_1 - \gamma_3a_3)(a_1 + a_2)\right. \\ &- KA_1^{-1}A_2^{-1}(\gamma_2a_1 + \gamma_3a_2)(a_3 - a_1) + a_4A_3^{-1}(a_3 + a_2)]\gamma \\ &+ KA_1^{-1}A_2^{-1}a_3\gamma_3(\gamma_3a_2 + \gamma_1a_2) \\ &- KA_1^{-1}A_2^{-1}a_1\gamma_1(a_1\gamma_2 + a_2\gamma_3) - a_4A_3^{-1}(\gamma_2a_3 + \gamma_1a_2) = 0 \end{aligned} \tag{7}$$

where

$$\begin{aligned} a_1 &= A_3^{-1}(x_2 - x_1) \\ a_2 &= A_2^{-1}x_1 \\ a_3 &= A_1^{-1}x_2 \\ a_4 &= \left[A_3^{-1}(\gamma_1 - \gamma_2)(x_2 - x_1) + (\gamma_1 - \gamma_3)x_1A_2^{-1}\right. \\ &\quad \left.+(\gamma_2 - \gamma_3)x_2A_1^{-1}\right] \end{aligned}$$

For systems containing two lipid components, 1:1 complex formation was assumed to be the explanation for deviation from the additivity rule. Model curves were constructed using calculated parameters such as equilibrium constants, molecular areas of the complexes, and interfacial tension of molecules and complexes. The accuracy of the models was verified by comparison to experimental results.

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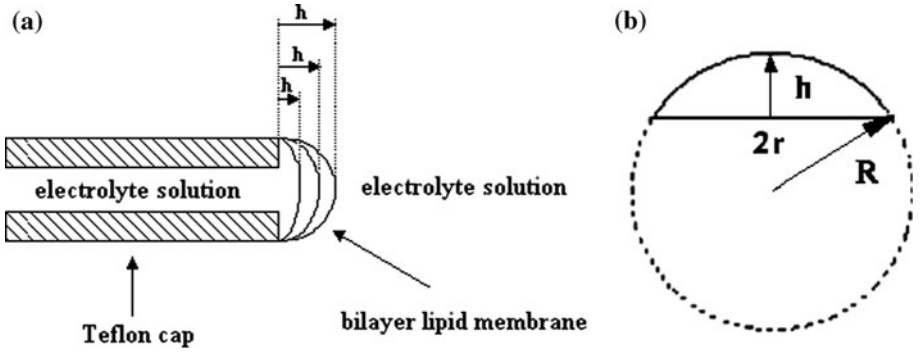
The radius of curvature, R, was determined using this value and the diameter of the Teflon element, corresponding to the diameter of the lipid cap and the convexity of the lipid membrane, which was presented below in drawing a and b:



Experimental

Measuring Apparatus and Measuring Procedures

The interfacial tension method is based on Young and Laplace's equation:

$$2\gamma = R\Delta p.$$

The interfacial tension, γ, in a lipid bilayer sample is determined by measuring the radius of curvature of the convex surface, R, formed when a pressure difference, Δp is applied across the bilayer [16].

The apparatus and measurement method were described in previous papers [13, 17]. The measurement vessel consists of two glass chambers separated by a mount holding a 1.5 mm diameter circular Teflon element axially pierced by a small orifice. Spherical membranes were formed by the Mueller–Rudin method [18] on the flat end of the Teflon element. Both chambers were filled with an electrolyte solution. The membrane-forming solution was introduced to the flat wall of the Teflon element using a micropipette, and pressure was applied to the left chamber using a manometer (VEB).

The convexity of the spherical cap was measured by means of a microscope with an objective equipped with a scale with 0.1-mm-interval scale marks. Therefore, the instrument readings of the lipid spherical cap were made with 0.05 mm precision. The convexity of the lipid membrane of the spherical cap, together with the Teflon element diameter corresponding to the lipid spherical cap diameter, yielded the radius of curvature. The measurement of the spherical cap was difficult because the spherical cap is

where R radius of curvature, r radius of the Teflon cap, h convexity of the lipid membrane.

Radius of curvature was calculated from equation: $R = r^2 + h^2/2h$.

We measured radius of curvature of bilayer lipid membrane and overpressure provoking the membrane convexity. Then we calculated the interfacial tension values from radius of curvature and pressure difference values according to Young and Laplace's equation.

The interfacial tension was measured on a freshly created lipid bilayer membrane 12–15 times. For each membrane about 10 instrument readings of the lipid spherical cap diameter, formed by pressure difference applied on both sides, were made. These measurements were made within the whole range, from the very low values of the lipid spherical cap diameter to those almost equal to the Teflon element radius. From all of instrument readings the arithmetic mean and standard deviation were enumerated. Measurements with preparation of the electrolyte solution were made 2–3 times in order to test the repeatability of these determinations. The experimental results are presented with error bars in the figures.

Reagents

The following reagents were used for the preparation of the membrane-forming solution:

1. Phosphatidylcholine (99%, Fluka) (fatty acid composition: 16:0 ~33%, 18:0 ~4%, 18:1 ~30%, 18:2 ~14%, 20:4 ~4%).

2. L-Valine (99.5%, Fluka);
3. L-Isoleucine (99.5%, Fluka);
4. L-Tyrosine (99.5%, Fluka);
5. Phenylalanine (99.5%, Fluka).

The molecular weights of the lecithin, valine, isoleucine, tyrosine, and phenylalanine were approximately 752.08, 117.15, 131.17, 181.19, and 165.19 g mol^{-1}, respectively.

The as-received phosphatidylcholine was purified by dissolving in chloroform and evaporating the solvent under argon. The stock membrane-forming solutions consisted of 20 mg cm^{-3} of the desired substances (PC, Val, Ile, Tyr, or Phe) in 20:1 *n*-decane:butanol. The solution containing the membrane components was not saturated and could therefore contain the components in any proportion. During membrane formation, the solvent was removed, leaving a membrane composed of lipids in the same ratio as the stock solution. Bilayer membranes were obtained as bubbles at the Teflon cap constituting a measuring vessel component. The use of *n*-decane as the solvent allows one to obtain membranes of thickness and capacity values similar to those of membranes formed of monolayers [19, 20]; there is almost no solvent retained in the bilayer. A small quantity of butanol added has a negligible effect on the interface tension values of the bilayers created; however, it considerably accelerates the formation of the membranes. The formation of the bilayers was monitored visually and electrically by measuring the membrane capacitance at low frequency (1 Hz). Capacity of the membranes increased with time after bilayers formation until a steady-state value was reached some 10–20 min later. The measurements were begun only after the low frequency capacitance was stable, increasing by, 1%/h. When the capacitance had stabilized, it was assumed that diffusion of solvent out of the bilayer was complete, although some *n*-decane molecules would remain dissolved in the membrane interior. The bilayers area was determined with a microscope with a micrometer scale built into the lens.

The electrolyte solution contained 0.1 M potassium chloride and was prepared using triple-distilled water and KCl produced by POCh (Poland). The KCl was calcined to remove any organic impurities.

All solvents were chromatographic standard grade. The *n*-decane was purchased from Merck and the chloroform and butanol were obtained from Aldrich.

All experiments were carried out at 293 ± 2 K.

Results and Discussion

The effect of the presence of amino acids on interfacial tension of the membranes formed from PC was studied. The dependence of interfacial tension of the lipid membrane as a function of composition was studied at room temperature (293 ± 2 K) in all the feasible concentration range. The interfacial tension values reported in this paper refer to the two sides of bilayer membrane surface area unit.

Figure 1 contains a graph of $(\gamma - \gamma_1)x_1$ versus $(\gamma_2 - \gamma)x_2$ for the four systems PC–Val (Fig. 1a), PC–Ile (Fig. 1b), PC–Tyr (Fig. 1c), and PC–Phe (Fig. 1d). According to Eq. 2, when the membrane components do not interact these functions should yield straight lines. This is clearly not the case, which suggests that a complex or other structure exists in PC–Val, PC–Ile, PC–Tyr, and PC–Phe bilayers. Because the use of Eq. 3 presupposes the existence of 1:1 complexes, our initial assumption was that the complexes formed were 1:1. The interfacial tension of the lipid membrane was studied over a wide range of lipid compositions.

Phosphatidylcholine–valine complex

Equation 2 predicts that in the absence of interactions, the plot of Fig. 1a should yield a straight line. The nonlinear nature of the plot indicates some form of interaction between phosphatidylcholine and valine. Such interactions in phosphatidylcholine–amino acid systems in monolayer can be explained in terms of complexes [21]. The 1:1 complex is formed in the initial stage of complexation, followed by other compositions in subsequent stages. In our case, an equation derived to describe the equilibrium of 1:1 complex formation was sufficient for the entire concentration range.

Figure 2a depicts the interfacial tension of a PC–Val membrane as a function of valine mole fraction. The dependences of interfacial tension of lipid membranes formed from the PC–Val system was executed in the function of the composition to 60% of the valine contents, because only to such contents of component 2 (valine) with lecithin were the forming bilayer membrane.

The interfacial tension value of pure lecithin membrane (component 1), γ_1 was measured directly and presented earlier [13], which is equal 1.62×10^{-3} N m^{-1}. There is no accurate literature data on interfacial tension values for the pure amino acid (valine, isoleucine, tyrosine, and phenylalanine), because these components are not creating the bilayer membrane. However, in order to characterize the course of the experimental curves, the γ_2 value for the pure components are necessary, which will be used for calculation. In this case, the interfacial tension hypothetical values for membranes built from amino acids were determined adjusting the experimental curve with the polynomial of the other mark extrapolating the $x_2 = 1$ value. An example of this extrapolation for valine membrane is presented in Fig. 3. The interfacial tension values obtained in this way for pure valine, isoleucine, tyrosine, and phenylalanine are

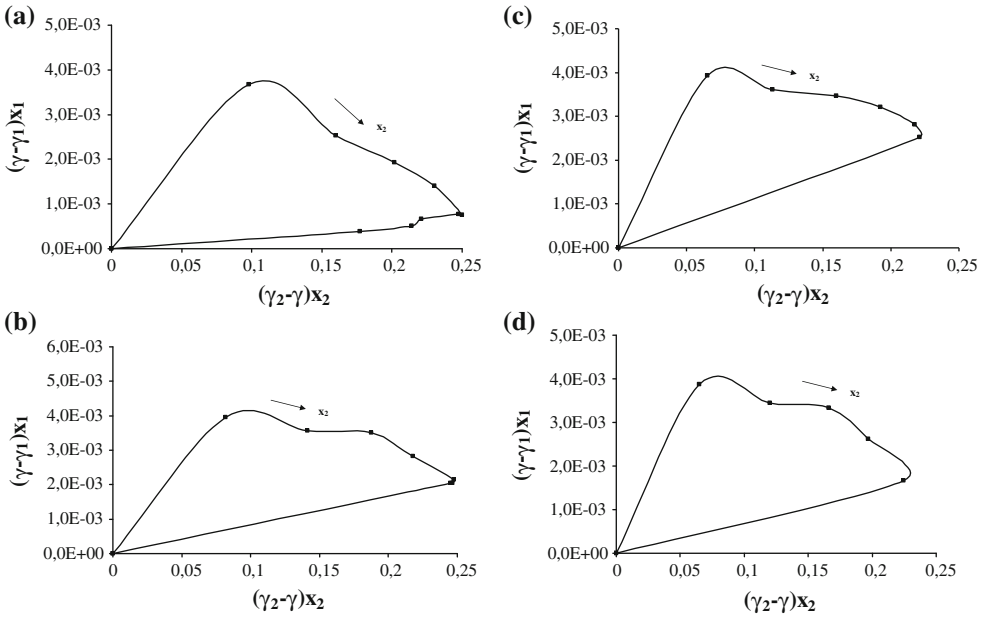


Fig. 1 Graph of Eq. 2 for phosphatidylcholine–valine (**a**), phosphatidylcholine–isoleucine (**b**), phosphatidylcholine–tyrosine (**c**), and phosphatidylcholine–phenylalanine (**d**), where x_2 is the mole fraction of component 2 (valine, isoleucine, tyrosine, phenylalanine, respectively)

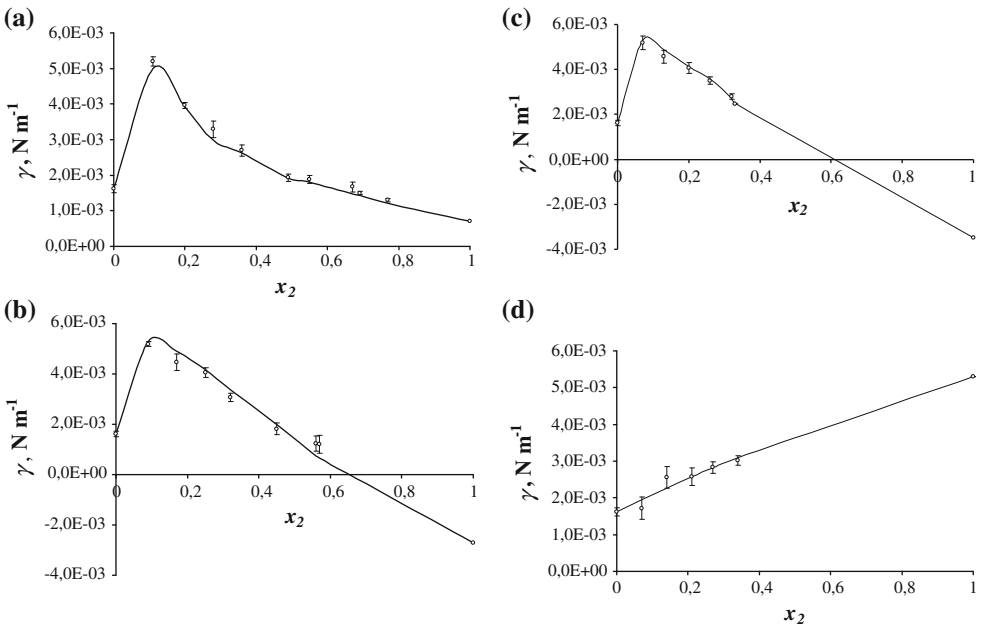


Fig. 2 The interfacial tension γ of phosphatidylcholine–valine (**a**), phosphatidylcholine–isoleucine (**b**), phosphatidylcholine–tyrosine (**c**), and phosphatidylcholine–phenylalanine (**d**), as a function of mole fraction x_2 of component 2 (the experimental values are marked by *points* and the theoretical values are indicated by the *curve*)

equal to 7.0×10^{-4}, -2.7×10^{-3}, -3.5×10^{-3}, and 5.3×10^{-3} N m^{-1}, respectively. Negative values of interfacial tension for membrane built from pure isoleucine and tyrosine are pointing to the fact that it is not possible to create the bilayer membrane from pure amino acid. Thermodynamic potential for this bilayer would have a negative value, i.e., the bilayer is not forming.

The valine and phenylalanine membrane interfacial tension value is positive. However, it is not possible to create a bilayer lipid membrane from the pure component because the forming solution above 60% of valine or 25% of phenylalanine was granulated in the solution.

Based on the literature, an assumption was made that a 1:1 complex (termed compound 3) formed the most prevalent structure and was characterized by a maximal stability constant K [15]. Given this assumption, the dependence of interfacial tension on the composition of the membrane-forming solution is described by Eq. 3. The interfacial tensions of membranes formed from the pure components were experimentally or theoretically determined. The constants B_1, B_2 and γ_3 were determined assuming that the value of the stability constant for the PC–Val complex was sufficient with respect to the simplified Eq. 3 to Eqs. 4 and 5.

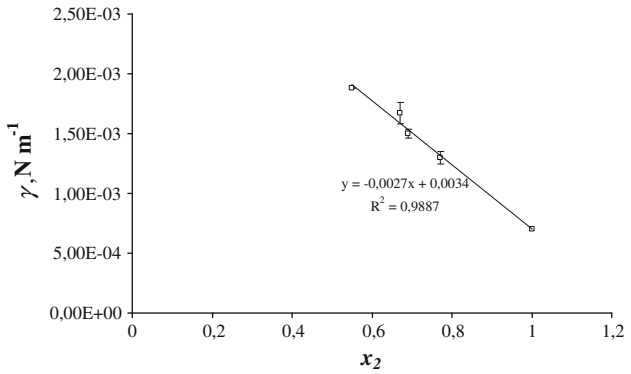


Fig. 3 A plot illustrating the hypothetical interfacial tension values for valine membrane calculation

Graphs of functions (4—denoted by 1) and (5—denoted by 2) are presented in Fig. 4. The values of B_1 (0.353) and B_2 (1.505) were determined from the slopes of the lines. The intersections of the straight lines with the ordinate provide $-B_1\gamma_3$ and $-B_2\gamma_3$, which can be used to determine γ_3, the interfacial tension of the PC–Val complex. The mean value obtained in this way was 2.04×10^{-3} N m^{-1}.

Determining the interfacial tension as a function of composition enabled the calculation of surface concentrations for membranes formed of pure components. At least one of these calculations is necessary to determine

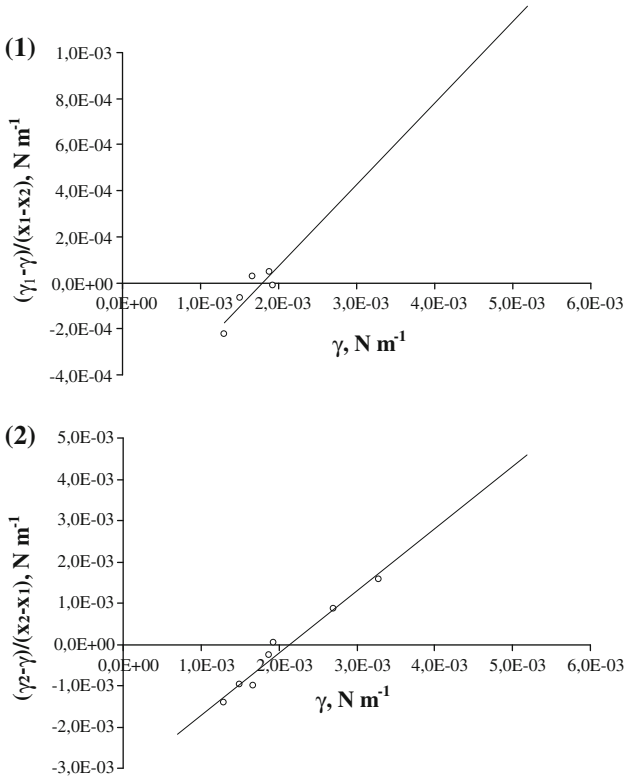


Fig. 4 A plot illustrating Eq. 4 (**1**) and Eq. 5 (**2**) for calculating the parameters B_1, B_2, γ_3 (for phosphatidylcholine–valine system)

the value of A_3^{-1}. The surface areas occupied by phosphatidylcholine and valine are 85 Å^2 mol^{-1} [17] and 155 Å^2 mol^{-1} (accessible surface area calculated for the residue X in the tripeptide G–X–G [22]), respectively.

The surface concentrations of phosphatidylcholine and valine in membranes of pure components, calculated from surface area presented above, are 1.96×10^{-6} and 1.07×10^{-6} mol m^{-2}. Knowing A_1^{-1} and A_2^{-1} as well as B_1 and B_2, the surface concentration of a membrane composed of PC–Val complex can be determined. The resulting surface concentration value A_3^{-1} for the PC–Val complex was 6.89×10^{-7} mol m^{-2}. From this it was possible to determine that the area occupied by one PC–Val complex is approximately 241 Å^2 mol^{-1}. The surface area value obtained in this way is higher than the area of a PC molecule, (85 Å^2 mol^{-1}), but is almost equal the sum of the areas of phosphatidylcholine and valine (240 Å^2 mol^{-1}).

The stability constant of the phosphatidylcholine–valine complex was determined from Eq. 6 by setting $x_1 = x_2 = 0.5$. The stability constant was 1.32×10^8 m^2 mol^{-1}. Knowing the stability constant value, the complex formation energy (Gibbs free energy) of a membrane composed of PC–Val complex can be determined. This value is equal -49.02 ± 1.47 kJ mol^{-1}.

Phosphatidylcholine–isoleucine, phosphatidylcholine–tyrosine, phosphatidylcholine–phenylalanine complexes

The dependence of lipid membrane interfacial tension on composition for PC–Ile, PC–Tyr, and PC–Phe systems were studied over a possible concentration range. The results are depicted in Fig. 2b–d. The dependences of interfacial tension of lipid membranes formed from the PC–Ile, PC–Tyr, and PC–Phe systems were executed in the function of the composition to 41% of the isoleucine contents (to 26% of the tyrosine and to 25% of the phenylalanine), because only to such contents of component 2 (isoleucine, tyrosine, phenylalanine) with lecithin were the forming bilayer membrane.

The values of γ_3 for the PC–Ile, PC–Tyr (1.91×10^{-3}, 1.75×10^{-3} N m^{-1}), and PC–Phe (3.69×10^{-3} N m^{-1}) complexes were calculated using Eqs. 4 and 5. Equations 4 and 5 could also be used to calculate the surface concentrations per unit area of membranes formed entirely from PC–Ile, PC–Tyr, and PC–Phe complexes (the surface area occupied by isoleucine, tyrosine, and phenylalanine are 175, 230, and 110 Å^2 mol^{-1}, respectively [22] and the surface concentration calculated for pure isoleucine, tyrosine, and phenylalanine membranes are 9.49×10^{-7}, 7.22×10^{-7}, and 1.51×10^{-6} mol m^{-2}, respectively). From these values it is possible to determine the areas

Table 1 Selected physicochemical parameters for four complexes: phosphatidylcholine–valine (PC–Val), phosphatidylcholine–isoleucine (PC–Ile), phosphatidylcholine–tyrosine (PC–Tyr), and phosphatidylcholine–phenylalanine (PC–Phe)

Examined system	Surface area occupied by one molecule of complex ($Å^2$ $molecule^{-1}$)	Stability constant of examined complex (m^2 mol^{-1})	Complex formation energy (Gibbs free energy) (kJ mol^{-1})
PC–Val	241 ± 2.41	1.32×10^8	−49.02 ± 1.47
PC–Ile	310 ± 3.10	1.97×10^7	−44.03 ± 1.32
PC–Tyr	398 ± 3.99	5.36×10^7	−46.97 ± 1.39
PC–Phe	219 ± 2.19	1.29×10^5	−30.85 ± 0.93

occupied by PC–Ile, PC–Tyr, and PC–Phe complexes, which are 310, 398, and 219 $Å^2$ mol^{-1}. The surface area values obtained in this way are higher than the area of a PC molecule, but are almost equal the sum of the areas of phosphatidylcholine and amino acid.

The stability constants of the PC–Ile, PC–Tyr, and PC–Phe complexes were determined using Eq. 6. The stability constant of PC–Ile complex is 1.97×10^7 m^2 mol^{-1}, and the constants PC–Tyr and PC–Phe are 5.36×10^7 and 1.29×10^5 m^2 mol^{-1}. It should be emphasized that the stability constant of phosphatidylcholine–tyrosine and phosphatidylcholine–phenylalanine are higher for complexes in bilayers than in the same systems in monolayer ($K = 6.04 \times 10^5$ m^2 mol^{-1} for PC–Tyr and $K = 1.39 \times 10^5$ m^2 mol^{-1} for PC–Phe complexes) [21]. A monolayer is a two-dimensional system forming a plane at the air/water interface, while a bilayer possesses a third dimension, and is additionally stabilized by hydrophobic interactions between the hydrocarbon chains.

Knowing the stability constant values, the complex formation energy (Gibbs free energy) of a membrane composed of PC–Ile, PC–Tyr, and PC–Phe complexes can be determined. These values are equal −44.03 ± 1.32, −46.97 ± 1.39, and −30.85 ± 0.93 kJ mol^{-1}, respectively.

The experimental values in Fig. 2b, c are marked by points, and the theoretical ones obtained from Eq. 7 are marked by lines. It can be seen from these figures that the agreement between experimental and theoretical points are good, which verifies the assumption of a formation of 1:1 PC–Ile, PC–Tyr, and PC–Phe complexes in the lipid membranes.

Table 1 lists several physicochemical parameters for membranes containing PC–Ile, PC–Tyr and PC–Phe complexes.

The analysis of the results presented in Table 1 leads to the following conclusions:

1. The stability constant of the PC–Val complex is 1.32×10^8 m^2 mol^{-1}, whereas the stability constant of the PC–Ile, PC–Tyr, and PC–Phe complexes are 1.97×10^7, 5.36×10^7, and 1.29×10^5 m^2 mol^{-1}, respectively. These values are relatively high, providing additional support for the prevalence of 1:1 complexes in mixed bilayers. These values also confirm that the assumption used to simplify Eq. 1 was correct. This paper contains the first report of stability constants for PC–Val, PC–Ile, PC–Tyr, and PC–Phe complexes.
2. The experimentally obtained value for the area occupied by the PC–Val, PC–Ile, PC–Tyr, and PC–Phe complexes are 241, 310, 398, and 219 $Å^2$, respectively.
3. The complex formation energy (Gibbs free energy) values for the PC–Val, PC–Ile, PC–Tyr, and PC–Phe complexes are −49.02 ± 1.47, −44.03 ± 1.32, −46.97 ± 1.39, and −30.85 ± 0.93 kJ mol^{-1}, respectively.
4. Good agreement between the experimental and theoretical points verifies the assumption of only 1:1 complex in the lipid membrane. The lack of variation between theoretical and experimental points indicates that our theoretical model (presented in the "Theory" section) is sufficient to describe the interaction in phosphatidylcholine–amino acids systems. The agreement between the experimental results and model predictions for the PC–Val, PC–Ile, PC–Tyr, and PC–Phe membranes justifies the statement that other complexes do not represent a significant component of these systems.
5. The mathematically derived and experimentally confirmed results presented here are of great importance for the interpretation of phenomena occurring in lipid bilayers. These results can help lead to a better understanding of the physical properties of biological membranes. The simple and very interesting methods proposed in this paper and in earlier studies [11–14, 23] may be used with success to determine the equilibrium constant values of 1:1 lipid–lipid, lipid–cholesterol, lipid–fatty acid, lipid–amine, and lipid–amino acid systems.

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