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The study of the interaction of a model α-helical peptide with lipid bilayers and monolayers

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Abstract

We studied the interaction of the α-helical peptide acetyl–Lys_2–Leu_{24}–Lys_2–amide (L_{24}) with tethered bilayer lipid membranes (tBLM) and lipid monolayers formed at an air–water interface. The interaction of L_{24} with tBLM resulted in adsorption of the peptide to the surface of the bilayer, characterized by a binding constant $K_c = 2.4 \pm 0.6\ \mu M^{-1}$. The peptide L_{24} an induced decrease of the elasticity modulus of the tBLM in a direction perpendicular to the membrane surface, $E_\perp$. The decrease of $E_\perp$ with increasing peptide concentration can be connected with a disordering effect of the peptide to the tBLM structure. The pure peptide formed a stable monolayer at the air/water interface. The pressure–area isotherms were characterized by a transition of the peptide monolayer, which probably corresponds of the partial intercalation of the α-helixes at higher surface pressure. Interaction of the peptide molecules with lipid monolayers resulted in an increase of the mean molecular area of phospholipids both in the gel and liquid crystalline states. With increasing peptide concentration, the temperature of the phase transition of the monolayer shifted toward lower temperatures. The analysis showed that the peptide–lipid monolayer is not an ideally miscible system and that the peptide molecules form aggregates in the monolayer.

Keywords: α-Helical peptide; Tethered bilayer lipid membranes; Phospholipid monolayers; Phase transitions; Aggregation

1. Introduction

Protein–lipid interactions play a crucial role in the formation of the stable structure of the biomembrane and in the functioning of proteins in a two-dimensional lipid matrix (see, e.g., Refs. [1,2]). So far, there is no full understanding of how proteins interact with lipids. Biophysical studies of protein–lipid interactions are usually performed on a model system composed of charged or uncharged α-helical peptides of different hydrophobic lengths and of lipid bilayers composed of phospholipids of different head groups and of different length of hydrocarbon chains. These peptides can span the lipid bilayers and thus model the behavior of integral proteins. Recent work by Harzer and Bechinger [3] showed the existence of a wide range of orientational distributions of α-helical peptides in a bilayer with orientation perpendicular or tilted to the membrane surface for peptides with large hydrophobic length up to orientation parallel to the membrane surface for shorter peptides.

The incorporation of hydrophobic peptides into the membrane should involve first adsorption to the bilayer surface and then incorporation into the hydrophobic interior. The adsorption of the peptide to the membrane surface can be studied using stable tethered bilayer lipid membranes (tBLM) by means of the electrostriction method, which allows determination of changes in membrane potential and in membrane compressibility. It is expected that peptide incorporation could cause changes in the area per phospholipid. This effect can be studied using lipid monolayers. Recent investigations have demonstrated the power of the method of lipid monolayers for the study the protein–lipid interactions [4,5]. In particular, the aggregation of several peptides, such as gramicidin A [6] or amphiphatic positively

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charged vector peptide [7], has been recently shown. Incorporation of these peptides also induced an increase of the mean molecular area of the phospholipids.

In this work, we studied the interaction of the α-helical peptide acetyl–K_2–L_{24}–K_2–amide (L_{24}) with tBLM and with lipid monolayers. L_{24} is composed of large hydrophobic part and two positive charges at its both ends [8,9]. We are interested to whether this peptide can incorporate into the lipid bilayer from buffer and whether the interaction with the membrane surface affects the physical and structural properties of tBLM and monolayers.

2. Materials and methods

2.1. Chemicals and preparation of bilayers and monolayers

tBLM were formed on a thin gold layer of a circular shape (diameter 0.7 mm) sputtered on a quartz plate. tBLM were composed of specially synthesized archaea analog 2,3-di-*O*-phytanyl-sn-glycerol-1-tetrathylene glycol-D,L-α-lipoic acid ester lipid (DPTL) [10] provided with a hydrophilic spacer and terminated by a cyclic disulfide group, that allowed chemisorption onto a gold support. The second monolayer was formed by fusion of unilamellar liposomes composed of diphytanoylphosphatidylcholine (DPhyPC) (Avanti Polar Lipids, USA) by mixtures with phosphatidylserine (PS) (Sigma, USA) in a molar ratio DPhyPC/PS = 10:1 (see Ref. [10] for more detail). In order to compare properties of tBLM with free standing bilayer lipid membranes (BLM), we studied also the electrostriction of BLM. BLM were formed from a DPhyPC/PS mixture (molar ratio DPhyPC/PS = 10:1) dissolved in *n*-decane (concentration 20 mg/ml) according to the method reported by Mueller et al. [11]. Lipid solution was spread across a circular hole (diameter 0.7 mm) in a Teflon wall separating two aqueous phase of Teflon chamber. The experiments have been performed in a 10 mM NaCl + 10 mM Tris–HCl buffer (pH 7.4).

Experiments on monolayers were performed with dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG) that were purchased from Sigma. All lipids were used as obtained. The peptide L_{24} has been synthesized as previously described [8,9]. In the case of tBLM, the L_{24} was dissolved in absolute ethanol in a concentration 1 mg/ml. This stock solution was then used for experiments with tBLM. Small amount of the peptide stock solution was added into the electrolyte in which tBLM was placed. The concentration of the ethanol in an electrolyte did not surpass 4% and has no effect on measured parameters. The lipid or lipid mixtures for experiments with monolayers were prepared at a concentration of 1 mg/ml in chloroform (Merck, Germany). The peptide L_{24} was first dissolved in 3:1 (v/v) chloroform–methanol at a concentration of 1 mg/ml. Appropriate amount of the stock solution was added to a small glass tube and dried under a nitrogen stream. After the complete evaporation of the solvent, pure chloroform was added at the final concentration of 1 mg/ml. The lipid/peptide mixtures were prepared at the desired molar ratios of 100:1, 50:1 and 20:1. Due to the tendency of the peptide to precipitate, as well as to avoid errors connected with changes of the peptide concentration caused by chloroform evaporation, each series of measurements was performed with a new peptide solution.

The protein, lipid or mixed protein–lipid monolayers were prepared by spreading of a small amount (totally approx. 50 μl) of the chloroform solution on the water subphase of a Langmuir–Blodgett trough using a microsyringe (Hamilton, USA). As a subphase, either deionised water (resistance>15 MΩ cm, ELIX 5, Millipore, El Paso, USA) or buffer (0.1. M NaCl + 10 mM Tris) (Sigma), pH 7.4 were used. Each monolayer was allowed to equilibrate for 15 min. This time was sufficient for solvent evaporation and monolayer reorganization.

2.2. Electrostriction method

The binding of the peptide to the tBLM has been studied by an electrostriction method [12] that allowed us to measure simultaneously the elasticity modulus in the direction perpendicular to the membrane plane, $E_\perp$, the electrical capacitance, C, and the transmembrane potential, Φ_m of the bilayer. Briefly, an alternating voltage of amplitude 50 mV was applied to the tBLM through reference Ag/AgCl electrode (the silver wire covered by AgCl was placed in an agar salt bridge). Due to the nonlinear dependence of capacitance on the voltage U [$C = C_0(1 + \alpha U^2)$, where C_0 is the capacitance at $U = 0$ and α is the electrostriction coefficient], the second and third current harmonics with frequencies $2f$, $3f$ and amplitudes I_2, I_3, respectively, will be generated in addition to the basic first current harmonic (frequency $f = 1$ kHz) of an amplitude I_1. The measurements of these amplitudes allowed us to determine the elasticity modulus:

$$E_\perp = 3C_s U_0^2 I_1/(4dI_3) \quad (1)$$

where C_s is the specific capacitance of the membrane ($C_s = C/A$, A is the membrane area) and U_0 is amplitude of the ac voltage and d is the thickness of the hydrophobic part of the membrane. The surface potential difference can be determined as follows. In contrast with symmetrical free standing BLM for which the open circuit potential (OCP) is 0, for tethered membrane there is nonzero OCP. Therefore, for transmembrane potential, Φ_m, we should write:

$$\Phi_m = -U_1 + U_0 I_2/(4I_3) \quad (2)$$

where U_1 is the externally applied potential to the tBLM [12]. We should note that externally applied dc potential does not contribute to the calculation of the value of $E_\perp$. It has been analyzed in detail in Ref. [12]. The value Φ_m represents a sum of the Gouy–Chapman, U_{GC}, and dipole

potentials U_d ($\Phi_m = U_{GC} + U_d$). The membrane capacitance is determined by:

$$C = I_1/2\pi f U_0 \quad (3)$$

(see Refs. [12,13] for details of the method).

2.3. Langmuir–Blodgett trough

In these experiments, we used a computer-controlled trough 601M instrument (NIMA Technology, Coventry, UK). The trough was equipped with two simultaneously moveable barriers and a Wilhelmy pressure sensor Nima PS4. The maximum working area was 86 cm^2 and the minimum was 14 cm^2. All the monolayers were compressed at the constant speed of 15 cm^2/min. The LB trough was thermostated by a water-circulating bath using a LAUDA E200 thermostat (Koenigshofen, Germany) with an accuracy of 0.05 °C.

3. Results and discussion

3.1. Adsorption of L_{24} to the tBLM

In the first series of experiments, we studied the binding of L_{24} to tBLM composed of a mixture of DphyPC and PS in a molar ratio 10:1. The elasticity modulus of pure tBLM was $E_\perp = (2.0 \pm 0.6) \times 10^7$ Pa (seven membranes). This value is significantly higher than that characteristic for solvent containing free standing BLM: $E_\perp = (3.5 \pm 0.8) \times 10^6$ Pa (five membranes). The specific capacitance of the tBLM was $C_s = 0.76 \pm 0.05$ μF/cm^2, which corresponds to the thickness of hydrophobic part of the tBLM of approx. 2.45 nm, which is comparable with the thickness of solvent-free membranes composed of phosphatidylcholines. The specific capacitance of BLM was lower: 0.4 ± 0.1 μF/cm^2. The differences between the $E_\perp$ and C_s for tBLM and BLM are connected with the presence of the solvent in the BLM, which makes the membranes more thick and softer (see Ref. [12] for more details). tBLM were rather stable and we did not observe substantial changes of elasticity modulus following application of external dc voltages in an interval from −500 to +500 mV. However, we observed minimum at the capacitance around −350 mV that can be connected with the substantial asymmetry of tBLM. Due to principal asymmetry of tBLM, the determination of surface potentials, i.e., U_{GC} and U_d, is rather complicated. For the lipid metal interface both the dipole potential (dependent on the orientation of the lipid head groups) and the fixed charge potential (i.e., U_{GC}) will be different from those at the lipid/water interface. The fixed charge potential at the lipid/water interface has been described using the Gouy–Chapman theory to account for the effects of screening by counterions from aqueous phase. This concept is not valid at the lipid/metal interface, and description based on the Stern model might be more appropriate [14]. Our experimental results showed that the minimum in the capacitance vs. voltage curve lies at about −350 mV; that is, this corresponds to existence of 350 mV surface potential difference across tBLM. Given that the fixed charge potential at the aqueous interface of tBLM in 10 mM NaCl is only −66.5 mV (determined by shielding experiment [13]), the major source of this surface potential is probably change of the dipole potential at the lipid/metal interface and the presence of OCP (this value was approx. 380–450 mV vs. Ag/AgCl with positive terminal on a gold working electrode). This result is rather interesting considering the fact that the head groups of DPTL are not directly adjacent to the metal surface, like sBLM [12], but are connected to the gold via approx. 2-nm-long polar spacer. That is, the counterions should be presented between gold and DPTL monolayer. The asymmetry between two monolayers of tBLM should, however, exist particularly due to the fixed orientation of the DPTL head groups to the gold that may influence the dipole potential of the inner, DPTL monolayer.

The interaction of L_{24} with the tBLM containing negatively charged PS resulted in stepwise neutralization of the surface charge and decrease of the surface potential. However, the changes of transmembrane potential $\Delta\Phi_m = \Phi_{mc} - \Phi_{m0}$ increase with increasing concentration of the peptide (Φ_{m0} is the initial transmembrane potential prior addition of the peptide and Φ_{mc} is the transmembrane potential at certain concentration of the peptide). This is reflected on the Fig. 1a, where the plot of the changes of transmembrane potential as a function of the concentration of L_{24} is presented. The changes of the transmembrane potential have been presented in order to avoid possible inaccuracy in consideration of the effects of external potentials (i.e., OCP potential) and tBLM asymmetry. From Fig. 1a, we can see that above a peptide concentration of 2 μM, the saturation of the changes of surface potential starts. The plot of $\Delta\Phi_m$ vs. peptide concentration can be fitted by a Langmuir isotherm: $\Delta\Phi_m = \Delta(\Phi_m)_{max} c/(c + K_c)$, where $\Delta(\Phi_m)_{max}$ is the maximal change of the potential, K_c is the binding constant and c is the bulk concentration of L_{24}. From the fit, we obtained $K_c = 2.4 \pm 0.6$ μM^{-1}. The obtained binding constant is comparable with that reported for binding of some amphiphilic fluorescence probes with the membrane [15]. Thus, the binding of the L_{24} with the tBLM surface is rather strong. It is particularly connected with the already mentioned negative surface charge of tBLM and by the presence of a total of four positive charges of each peptide molecule. Considering the structural peculiarities of L_{24}, i.e., the peptide containing a long sequence of hydrophobic leucine residues capped at both the N- and C-termini with two positively charged, relatively polar lysine residues, the incorporation of the peptide into the hydrophobic part of the tBLM is not favorable. This was also suggested from the results on the measurement of surface potential. If the

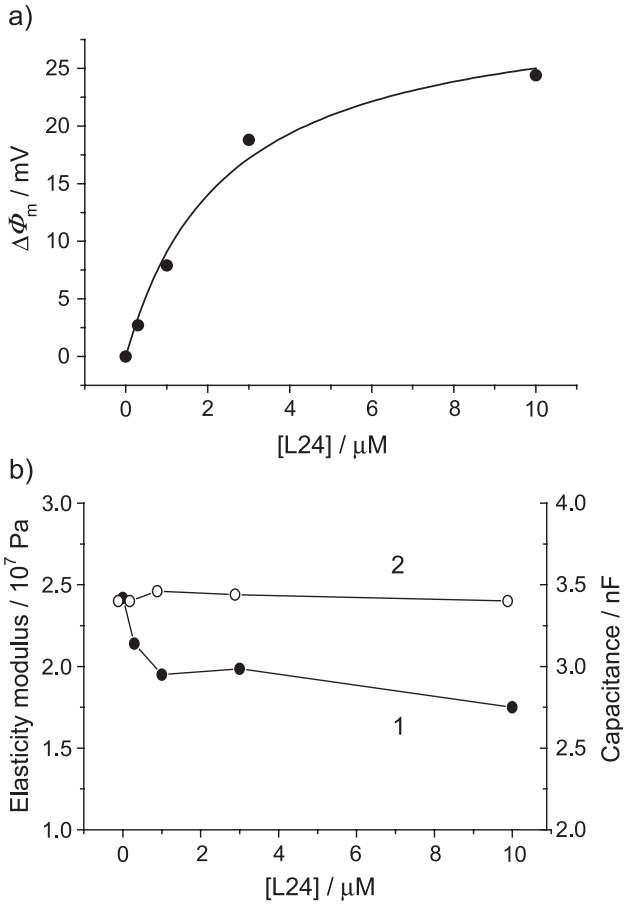

Fig. 1. (a) The plot of the changes of transmembrane potential $\Delta\Phi_m = \Phi_{mc} - \Phi_{m0}$ (Φ_{m0} is the initial transmembrane potential prior addition of the peptide and Φ_{mc} is the transmembrane potential at certain concentration of the peptide). (b) Elasticity modulus (1) and membrane capacitance (2) vs. concentration of L_{24}. The curve at Panel (a) represents the fit according to the Langmuir isotherm.

peptide is inserted into the hydrophobic interior of the membrane, the second positively charged terminus should appear on the opposite side of the membrane. In this case, we should observe an increase of the transmembrane potential, which was not the case. The interaction of the peptide with tBLM is accompanied by decrease of the elasticity modulus $E_\perp$, while electrical capacitance, C, of the tBLM does not change significantly. This is evident from the Fig. 1b, where the plot of the $E_\perp$ and C as a function of the peptide concentration is presented, The α-helical peptide seems rather flexible. For example, molecular modeling studies suggested a bent helix [16]. Therefore, it is reasonable to assume that the hydrophobic part of the peptide could be partially inserted into the core of tBLM. The changes of elasticity modulus probably reflect this effect. The decrease of $E_\perp$ with increasing the peptide concentration can be connected with a disordering effect of the peptide to the tBLM structure. Particularly, the peptide could cause an increase of the area per phospholipid molecule that may result in an increase of the conformational freedom of the hydrophobic chains of the lipids. Thus, the compressibility of the membrane (i.e., $1/E_\perp$) will increase. In order to check this possibility, we performed experiments using lipid monolayers.

3.2. Monolayers of pure peptide

The pure peptide L_{24} forms stable monolayers on the water subphase, as seen in Fig. 2, where pressure–area isotherms for L_{24} on either deionised water or buffer subphase are presented. The isotherms are characterized by a structural transition of the peptide monolayer. The transition on the deionised water subphase is not as pronounced as that on the buffer subphase and starts at a higher surface pressure (13 mN/m compared to 10 mN/m for buffer). These transitions are probably connected with the changes of the packing density of the peptide. At higher surface pressure, the α-helixes could probably partially intercalate in analogy with similar effect observed for gramicidin A [6]. This phenomena is, however, not fully understood because the existence of positively charged ends of the peptide that should make this intercalation not favorable. On the other hand, the fact that the transition starts at lower pressure on buffer may be explained by a partial shielding of these positive charges by anions. Additional study, e.g., using AFM method, will be required for a better understanding of the geometry of the peptide at the air–water interface. The obtained molecular area of the peptide at the region above the transition of the monolayer is ~ 1.7 nm^2/molecule (1.70 nm^2 at the water and 1.65 nm^2 at a buffer), which is slightly lower then the geometrical cross sectional area of the peptide, approx. 1.77 nm^2 (see Ref. [7] for method of calculation). The collapse of the monolayer on the water subphase takes place at approx. 30 mN/m, which is slightly higher than that for monolayers formed on buffer (27.8 mN/m). This suggests a higher stability of peptide monolayers on the

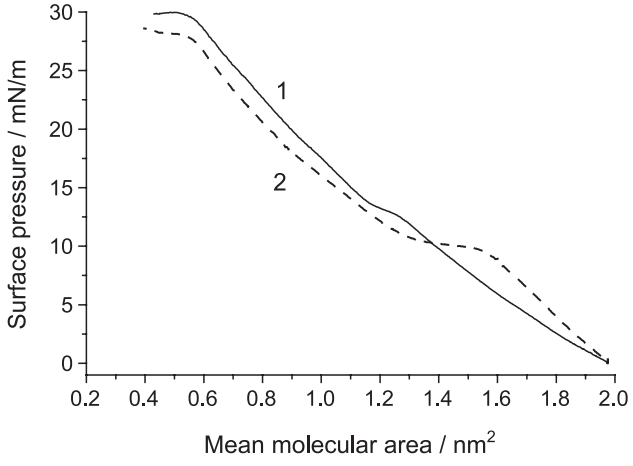

Fig. 2. π–A isotherms of pure peptide L_{24} at the different subphases: (1) deionised water; (2) buffer (0.1. M NaCl + 10 mM Tris, pH 7.4). T = 18 °C.

deionised water subphase in comparison with that on buffer.

We also analyzed possible desorption of the peptide molecules from the monolayer to the water subphase. For this purpose, we performed experiments in which the monolayer was first compressed to a pressure of 24 mN/m, i.e., below the collapse pressure. The monolayer was then reexpanded and compressed again. The second compression was characterized by only a negligible shift toward lower areas (results are not shown), probably due to a slight decrease of the number of peptide molecules in the monolayer or a decreased area caused by aggregation of the peptides. Thus, due to a negligible hysteresis, we can assume that there is no significant desorption of the peptide from the monolayer.

3.3. Mixed peptide/DMPC monolayers

The interaction of the peptide with the lipid monolayers can be studied by analyzing the thermodynamics of mixed peptide–phospholipid monolayers formed at an air–water interface. Saturated DMPC bilayers are characterized by a phase transition from gel to liquid crystalline state at approx. 24 °C [17]. Therefore, we had possibility also to analyze the peculiarities of the interaction of L_{24} with DMPC monolayers to different structural states.

The isotherms of DMPC monolayers at various temperatures are shown on Fig. 3a. They have typical shape characteristic for phosphatidylcholine monolayers [18,19]. It is easy to distinguish several regions that correspond to the different structural states of the monolayer. The liquid-expanded structure (L-E) turns into liquid-condensed state (L-C) at the surface pressure of ~ 7 mN/m. At the surface pressure ~ 34 mN/m, a second and much less significant plateau can be observed that corresponds to the transition from the L-C phase to the solid phase (S). This phase is accompanied by the lowering of the tilt of the chains which are now almost perpendicular to the membrane plane. The molecules in the S phase are so densely packed that further compressions (at approx. ~ 40 mN/m) causes the lost of the two dimensional structure and the monolayer collapses (C) (i.e., multilayer regions appear after the collapse). Extrapolation of the linear part of the isotherm at the S phase to the zero surface pressure gives a value 0.68 nm^2/molecule. This value is in good agreement with the mean molecular area of phosphatidylcholines with acyl chains of similar length [18,19]. The phase transition is accompanied by loss of the plateau at the surface pressure of ~ 6 mN/m. Indeed, the isotherms of the monolayers above the phase transition (at $T \geq 24$ °C) had no plateau at this surface pressure. The increase of temperature is accompanied by an increase of the mean molecular area with a sharp increase at the temperature of the phase transition of the phospholipid ($T = 24$ °C). Results are not shown but were in good agreements with that reported earlier [18].

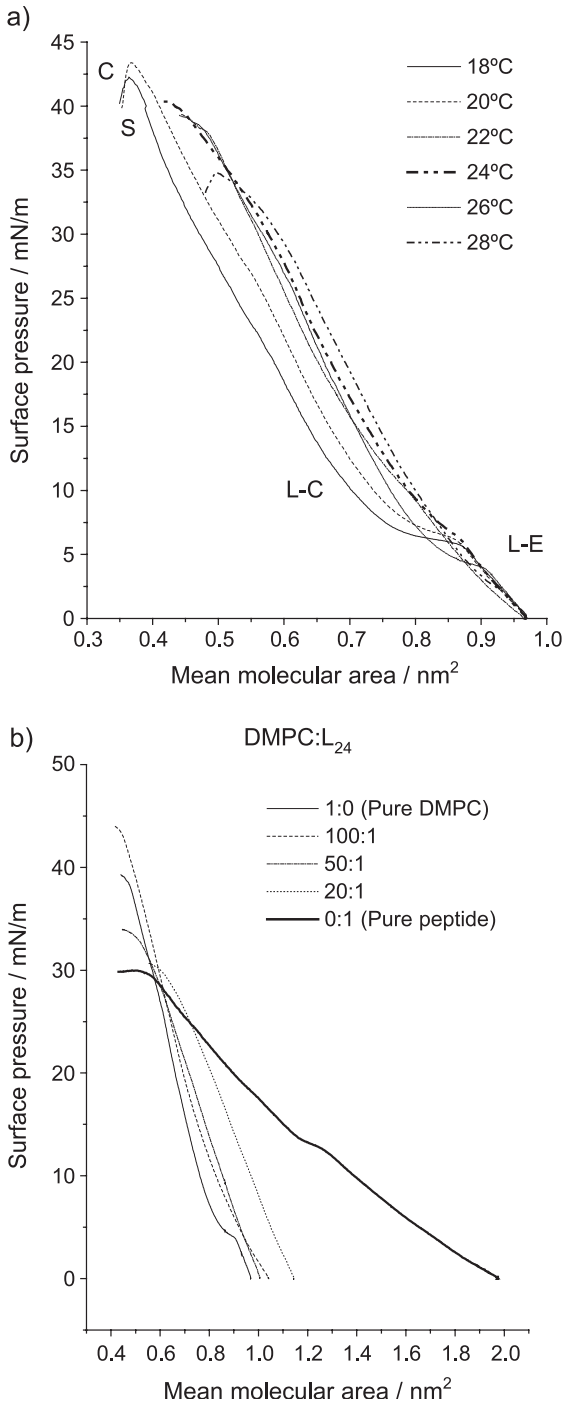

Fig. 3. π–A isotherms of lipid monolayers formed by (a) DMPC at the deionised water subphase at different temperatures [liquid expanded (L-E), liquid solid (L-C); solid regions (S), collapse (C)—for explanation see the text]. The temperature values are shown at the legend. (b) Mixed DMPC:L_{24} monolayers at different DMPC/peptide molar ratio at $T = 18$ °C. Subphase: deonised water.

The π–A isotherms for mixed DMPC/L_{24} monolayers are presented on Fig. 3b for various molar ratios of phospholipid and peptide at $T = 18$ °C, i.e., for DMPC monolayers at the gel state. For comparison, also the

isotherms for pure DMPC and pure peptide are presented. We can see that in comparison with pure peptide, the mixed isotherms are characterized by higher collapse pressure. This means that the mixed monolayers are more stable than those composed of pure peptide. We can also see that already at low contents of the peptide, the plateau characterizing the coexistence of the L-E and L-C phases (i.e., at $\pi = 7$ mN/m) disappeared. In general, above the surface pressure of 10 mN/m, the mean molecular area increases with increase in the content of the peptide molecules. The molecular area increased also at the liquid crystalline state of the monolayers as well as for both gel and liquid crystalline state of monolayers formed on a buffer subphase, i.e., containing 0.1 M NaCl (results are not shown). However, in the case of the buffer subphase, the transition region at 7 mN/m at the gel state of the monolayer was visible even at higher peptide content (DMPC:L_{24} = 20:1 mol/mol). This suggests a weaker interaction of the peptide with DMPC under these conditions and is in agreement with the results presented above for pure peptide monolayers on this subphase. With increasing the concentration of the peptide, we also observed a decrease in the temperature of the phase transition of the phospholipids, T_F (Fig. 4). The increase of the molecular area with an increased content of peptide and a decrease in the temperature of the phase transition of the monolayer indicates a disordering effect of the peptide molecules on a lipid monolayer structure. The obtained results correlate well with those obtained by the electrostriction method (see above).

The analysis of the miscibility of proteins and lipids can provide additional insight to the mechanism of the L_{24}–phospholipid interactions. In the case of ideal miscibility, the mean molecular area in a mixed monolayer (A_{12}) can be expressed as the sum of the mean molecular area of the pure components (A_1 and A_2 respectively) multiplied by their corresponding molar fraction X in a monolayer [5]:

$$A_{12} = XA_1 + (1 - X)A_2 \quad (4)$$

In the case of ideal miscibility, the plot of the mean molecular area at constant surface pressure as a function of the fraction of the corresponding component of the monolayer should be a straight line. Any deviation from linearity indicates changes in the miscibility of the monolayer components. In the analysis, we assume uniform orientation of the peptide relatively to the monolayer surface. Fig. 5a shows the dependence of the mean molecular area on the molar fraction of L_{24} molecules in DMPC monolayers formed on a water subphase at $T = 18$ °C at a surface pressure of 15 mN/m. We can see that the experimental points lie above the straight line, which

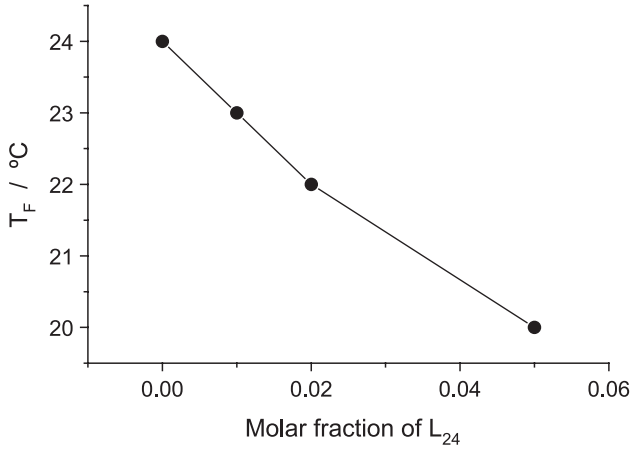

Fig. 4. The plot of the temperature of phase transition T_F of the lipid monolayer as a function of the content of the peptide L_{24}. Subphase: deionised water.

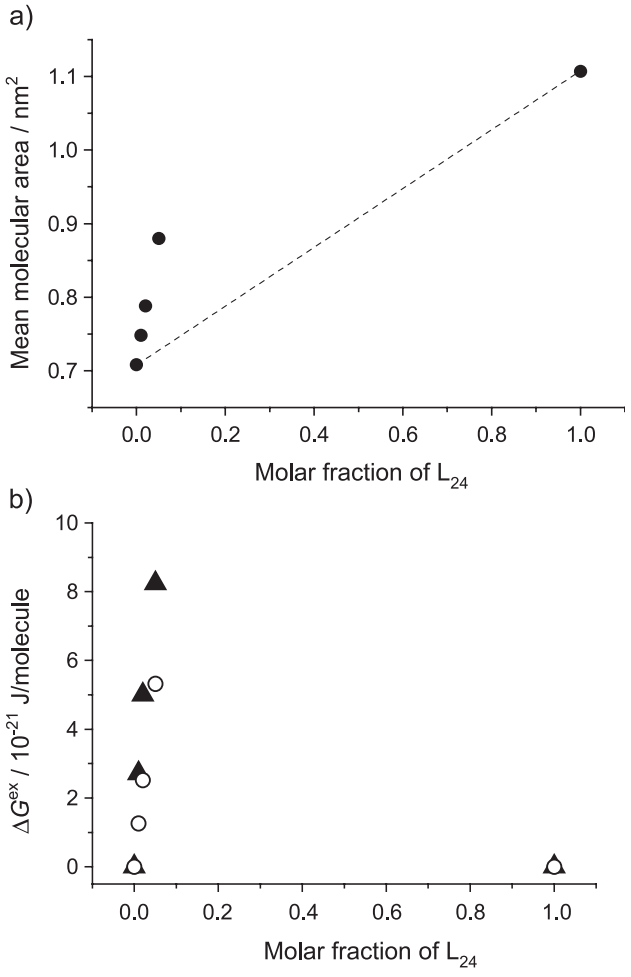

Fig. 5. (a) The plot of the mean molecular area as a function of the molar fraction of peptide for mixed DMPC/L_{24} monolayers. (b) The results of calculation of excess energy ΔG^{ex} by means of Eq. (3) as a function of the molar fraction of L_{24} for mixed phospholipid/L_{24} monolayers composed of DMPC (○) or DMPC–DMPG mixture in a molar ration 10:1 (▲), the level of integration π = 35 mN/m. The monolayers were formed on a deionised water subphase, T = 18 °C.

represents the dependence for ideally miscible components. This result clearly indicates that there is no miscibility between the lipids and the L_{24} molecules. Having these results, it is also possible to calculate the excess Gibbs energy, ΔG^{ex}, corresponding to the interaction between DMPC and L_{24} molecules. The calculation can be performed using Eq. (5) (see Ref. [5]):

$$\Delta G^{ex} = \int_0^{\pi} A_{12} d\pi - X \int_0^{\pi} A_1 d\pi - (1 - X) \int_0^{\pi} A_2 d\pi, \quad (5)$$

where the upper limit of calculation can be selected on the following assumption. It is generally accepted that lipid monolayers mimic the lipid bilayers at the surface pressure around 35 mN/m [20]. We therefore chose 35 mN/m as an upper limit of integration. The results of calculations are presented on Fig. 5b for mixed phospholipid/peptide monolayers composed either on DMPC or a mixture of DMPC/DMPG (molar ration 10:1). The presence of DMPG provided a certain negative surface charge of the monolayer that is characteristic for, e.g., bacterial cell membranes. We can see that for both phospholipid monolayers, the excess energy is positive. In addition, this energy is higher for mixed DMPC/DMPG monolayers in comparison with DMPC monolayers. The values of $\Delta G>0$ obtained means that the interaction between two components of the monolayer, i.e., lipids and proteins, is not favorable. This suggests that there is tendency for the formation of aggregates between the molecules of the same kind, i.e., the peptide. The tendency to peptide aggregation is characteristic for both liquid-expanded and liquid-condensed state of the monolayer.

The aggregation of the peptides in a bilayer is probably caused by the considerable mismatch between hydrophobic part of the peptide and the length of phospholipid hydrocarbon chains. Due to the fact that length of hydrophobic part of the peptide is much larger (3.1–3.2 nm according to the estimations using molecular models [17], than that of the lipids (1.71 and 1.14 nm, in gel and liquid crystalline state, respectively—i.e., calculating on the assumption that these values are half of the thickness of the bilayer [17]) as well as due to the positive charges of the peptide terminal groups, it is more favorable that the peptide molecules are oriented parallel to the monolayer surface. In the case of parallel orientation of the peptide, there is still mismatch due to the fact that the diameter of the α-helix is according to our experiments performed on monolayers approx 1.47 nm (see above), which is less then the length of hydrocarbon chain of phospholipids in a gel phase, but larger with that in a liquid crystalline state. In this orientation, both polar ends of the peptide could interact with the phospholipid head groups, while the hydrophobic part of the peptide will initiate repulsive forces both with the polar head group of the phospholipids as well as with water molecules. In order to minimize these repulsive forces, the aggregation of the peptides should be energetically favorable in a monolayer. Formations of oligomers could minimize the contact area between hydrophobic part of the peptide and the polar head groups of the phospholipids or with water molecules. The behavior of L_{24} in lipid monolayers is in considerable contrast with that in bilayers. In later case, the minimal mismatch between hydrophobic part of the peptide and the membrane thickness, as well as the positive charge of the terminals groups, protect the peptide from aggregation at physiological pH [21].

4. Conclusion

The results obtained suggest that L_{24} adsorbs to negatively charged lipid bilayers but presumably is only partially inserted into the membrane interior. Experiments on monolayers suggest that the peptide molecules form stable monolayers at an air–water interface. The phospholipids in a mixed monolayers caused further stabilization of these monolayers. The peptide molecules are predominantly organized in aggregates in the mixed phospholipid monolayers. However, the decrease of the temperature of phase transition of phospholipids, as well as increase of the mean molecular area with increasing the content of the peptide molecules in a mixed monolayers, suggest a disordering effect of the L_{24} on a phospholipid monolayers. We should note however, that the mixed monolayers studied cannot be exactly used for explanation of the behavior of the L_{24} in lipid bilayers. This is due to the different orientation of L_{24} in monolayers as well as to the considerable mismatch between hydrophobic part of the L_{24} and the hydrophobic chains of the phospholipids. However, the results obtained are in good agreement with that on tBLM obtained in the study of the peptide adsorption in respect to the explanation of the reason for the disordering effect of L_{24} on the membrane, i.e., by means of increase area per phospholipid in a BLM monolayer.

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