Self-Organization in Two-Dimensional Organic Molecular Systems (Applications in Biomembranes)

Július Cirák*

Department of Physics, Faculty of Electrical Engineering and Information Technology, Slovak University of Technology, Ilkovičova 3, 812 19 Bratislava, Slovak

Abstract: Formation of a monomolecular layer (the Langmuir monolayer) at the air/water interface is a self-organized process. On the basis of measurements of π - A isotherms, mechanical and thermodynamic properties of 2D molecular systems can be evaluated. The concept is applied to the study of molecular interactions in a binary system in biological membranes: L-α-dimyristoyl phosphatidylcholine (DMPC) and gramicidin A.

Keywords: Langmuir monolayer, surface pressure – area isotherms, excess Gibbs energy, DMPC, gramicidin A.

1. INTRODUCTION

Monomolecular insoluble layers on the surface of a liquid termed the Langmuir monolayers are formed as a result of a self-organized process. Organic molecules constituting the monolayer are amphiphilic and they spontaneously spread at the air/water interface. The water surface provides ideally planar and smooth (noncorugated) surface as a substrate. A pair of thermodynamic quantities, temperature and surface pressure, can be easily controlled; surface pressure by a moving barrier over the surface. Such mechanical compression, which is analogous to hydrostatic compression in 3D systems, is not available in other 2D systems and arrangements. In addition to this, the interaction of molecules in the monolayer can be systematically modified by the exchange of polar and nonpolar parts of surfactant molecules using wide synthetizing possibilities of organic chemistry (e.g. the chain length can be varied by nanometer steps) or by the change of pH or of ionic content of the subphase. The Langmuir monolayers are excellent model systems in membrane biophysics, because biological membranes can be considered as two superimposed weakly interacting lipid monolayers.

The effect of self-organization is a creating principle in biological membranes, to form a 2D fluid mosaic model. From topological and morphological point of view a membrane can be regarded as a 2D solution of integral proteins in a lipid layer. Protein molecules are not distributed randomly in their lipid environment but they form clusters in order to provide optimum lateral configuration of enzymes performing sequential enzymatic reactions, or several protein molecules in a selective ion channel to control charge carriers transport through the membrane. This is also an attractive approach in information processing technology, which is based on molecules as basic building blocks, and to assemble the molecules together in a controlled manner so as to create a structure with designed electronic function.

*Address correspondence to this author at the Department of Physics, Faculty of Electrical Engineering and Information Technology, Slovak University of Technology, Ilkovičova 3, 812 19 Bratislava, Slovak; Tel: 02/602 91 459; E-mail: julius.cirak@stuba.sk

This communication presents the study of two-component system: gramicidin A with lipids. Gramicidin, secreted by *Bacillus brevis,* is considered as a model peptide for studies of mechanisms of ion transport as well as mechanisms of protein-lipid interactions. Ion channels formed by gramicidin molecules behave like an ion-selective gate in FET transistors with switching sensitive to external conditions. Considering a different conformation between the open and the closed state of the ion channel one can expect the effect of the channels on physical properties of biomembranes.

2. TWO-DIMENSIONAL LATERAL DOMAINS IN LANGMUIR MONOLAYERS – THEORETICAL APPROACH

The Langmuir monolayer is a very suitable model for the study of self-assembly processes in two dimensions and molecular order parameters, in-plane as well as in normal direction.

In a two-component 2D molecular system, *i.e.* a monolayer consists of 2 different types of molecules, A and B, the distribution of molecules will depend on molecular interactions between the molecules of the same type (A – A, B – B) on one hand and between the molecules of different types (A – B) on the other hand. In our simple model we considered the molecular interaction A – A with the binding energy of E_V and non-interacting pairs B – B and A – B. If B molecules are homogeneously dispersed among A molecules and they form the orthogonal lattice, the excess of internal energy of the mixed molecular system over the original A system is equal to:

$$U_h = 4NE_V \quad (1)$$

where N is an overall number of B molecules. In case that B molecules are not evenly dispersed but they are assembled in 2D clusters – circular domains, the molecules of different types are in contact only along the circumference of the domain. If n is a number of B molecules constituting the domain it can be shown that the number of B molecules on the boundary is $\pi n^{1/2}$ and the excess of internal energy:

1874-3439/10 2010 Bentham Open

$$U_d = \pi n^{1/2} \frac{N}{n} E_V \quad (2)$$

Thus the formation of domains in a formerly homogenous system results in a decrease of the internal energy:

$$\Delta U = U_d - U_h = -NE_V \left(4 - \pi n^{-1/2}\right) \quad (3)$$

This is a monotonous function, which supports a tendency to creation of large domains, ultimately of one domain with all B molecules inside. But the entropy effect acts just opposite. Formation of domains brings about a decrease of entropy, which represents a positive contribution to the principal thermodynamic quantity of state – to Gibbs's energy

$$G = U + \pi A - TS \quad (4)$$

This equation is rewritten for a 2D system, πstands for the surface pressure and *A* is the area occupied by the molecules at the surface of water (in our model these quantities remain unchanged). Again if we consider the starting configuration with B molecules being evenly dispersed in the array of A molecules, according to Boltzmann's entropy equation:

$$S_h = k_B \ln W = k_B \ln N! \quad (5)$$

with *W* the multiplicity of the configuration.

If the domains consisting of n molecules of B type are formed:

$$S_d = k_B \ln \frac{N!}{(n!)^{N/n}} \quad (6)$$

The change of entropy accompanying the change in the configuration:

$$\Delta S = S_d - S_h = -k_B N (\ln n - 1) \quad (7)$$

and the change in Gibbs's energy is as follows:

$$\Delta G = -NE_V \left(4 - \pi n^{-1/2}\right) + k_B TN (\ln n - 1) \quad (8)$$

The final configuration will be characterized by minimal Gibbs's energy, therefore a differentiation with respect to the domain size will reveal the number of B molecules in one domain:

$$n = \left(\frac{\pi E_V}{2k_B T}\right)^2 \quad (9)$$

The exact treatment of the shape evaluation is beyond the aim of this study. Recently developed sophisticated laser – based technique, Brewster angle microscopy, can visualize such monolayer lateral domains (Fig. **1**).

3. MATERIALS AND METHODS

3.1. Chemicals

Dimyristoylphosphatidylcholine (DMPC) (MW = 678) was purchased from Avanti Polar Lipids (USA), Gramicidin A (gA) (MW = 2000) was from Sigma (USA). For preparation of monolayers, DMPC and gA were dissolved in chloroform at a concentration of 1 mg/ml and at various molar ratios. As a subphase, bidistilled and deionised water (15 MΩ.cm, ELIX 5, Millipore, USA) was used. DMPC, gA or mixed DMPC/gA monolayers were prepared by casting a small amount of solutions (approx. 25 μl) over the water subphase of the Langmuir – Blodgett trough using microsyringe (Hamilton, USA).

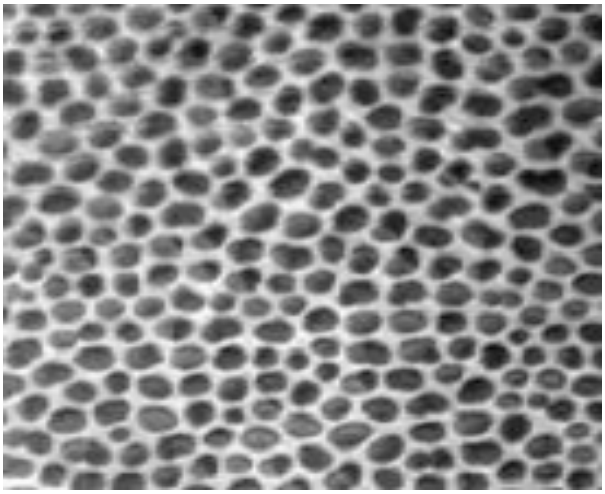

Fig. (1). Brewster angle microscopy of circular liquid-condensed domains in amphiphilic monolayers at the air/water interface (the depicted area is ca. 50 μm x 50 μm).

3.2. Experimental Methods

Computer – controlled Langmuir trough model 611M (NIMA Technology, Coventry, UK) was used for monolayer experiments. The maximum and minimum working area of the trough were 600 cm^2 and 75 cm^2, respectively. The surface tension (p) of the monolayer was measured by the Wilhelmy method with accuracy of ±0.5 mN/m. The monolayer was allowed to equilibrate and the solvent to evaporate for 15 minutes. This time was found sufficient for evaporation of chloroform and stabilization of the monolayer. All monolayers were compressed at the constant speed of 5 cm^2/min, which corresponds to 0.17 $Å^2$/s per molecule. The trough was thermostated by a recirculating cooler FL 300 (Julabo Labortechnik, Germany) with an accuracy of 0.1 ºC. The experiments were carried out at temperatures of 20 ºC, 24 ºC, and 28 ºC, i.e. in the gel phase of DMPC, at the phase transition, and in the liquid crystalline phase.

4. RESULTS

The fundamental characteristic description of a monolayer is usually in terms of its surface pressure – area curve, i.e. the relationship between the surface pressure observed and the area occupied on the liquid surface by the molecules of the film. This π - A dependence provides direct evidence on the phase transformations during the isothermal compression in 2D molecular system.

Surface pressure – area isotherms of DMPC and gA monolayers as well as of their binary mixtures are presented in Fig. (**2**). Pressure – area isotherms have typical shape as reported earlier for pure DMPC, gA or their mixtures [1]. We can see that monolayers for pure DMPC are in liquid – expanded state (LE) at the high area per molecule (A > 0.8 nm^2) and at the pressure surface pressure $\pi \approx 8$mN/m turns into the liquid – condensed state (LC). In a gel state of DMPC (T=20 ºC) a plateau is observed at $\pi \approx 25$mN/m. This

plateau is less expressed at T=24°C that corresponds to phase transition of DMPC (not shown in this figure) and disappears in a liquid-crystalline state of DMPC (T=28 ° C). The area – pressure isotherm for pure gA is also in agreement with previously reported [2]. These isotherms are not sensitive to temperature in the range T=20–28°C and are characterized by typical plateau at $\pi \approx 14$mN/m. The label "condensed" is used to denote all the states of the lipid monolayer with the hydrocarbon chains aligned, in contrast with expanded states where the chains are conformationally disordered. The transition from the solid state to the liquid – condensed state occurs here at approx. at 25 mN/m as a typical kink on the isotherm, with the compressibility decreasing further after the kink. This is a transition from tilted to untilted form [3].

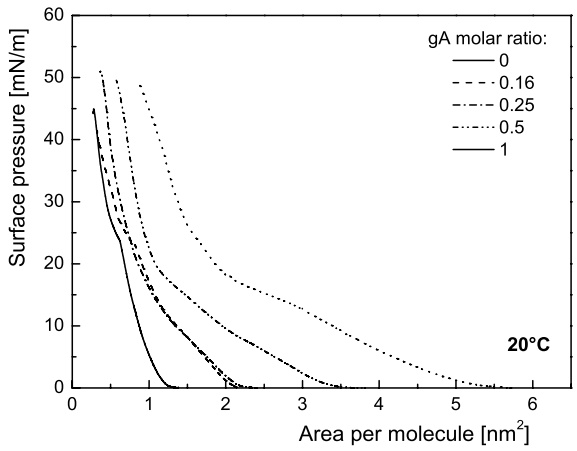

Fig. (2). Pressure-area compression isotherms of gA/DMPC mixed monolayers and pure components at an air-water interface. Curves correspond to various molar fraction of gA (see insets in the figures). 0 means pure DMPC and 1 is pure gA. The isotherms shown were recorded at 20 ^{0}C.

The miscibility of two compounds in a mixed monolayer at the water-air interface can be deduced as a result of the behaviour of molecular areas as a function of the mixture composition at constant surface pressure and temperature. Positive or negative deviations from an ideal dependence of the excess area as a function of gA/DMPC ratio, characterize repulsive or attractive mutual interactions between the monolayer components: gramicidin A and DMPC molecules [4, 5]. For this reason, monolayers in which components are either immiscible or if they behave like an ideal mixture, the excess area of the mixture, DA, defined as

$$\Delta A = A_{12} - x_1 A_1 - x_2 A_2 \tag{10}$$

is equal to zero. A_{12} is the molecular area in the mixed monolayer at temperature T and surface pressure π, whereas A_1 and A_2 are molecular areas in the two single component monolayer and x_1 and x_2 are molar ratios of the pure components in the mixture ($x_1 + x_2 = 1$). Excess areas of the mixture DMPC/gA molar ratio dependences are shown in Fig. (**3**) for DMPC monolayers in a gel (20°C).

It is seen from the figure, that the excess area isotherms depend not only on the temperature, but also on the surface pressure. For a low temperature experiment (gel state of DMPC) a negative excess area for all molar ratios of gA/DMPC mixtures was observed at constant surface pressure 30 mN/m. This suggests high miscibility of gA and DMPC molecules without creation of domains of pure components. However, at lower surface pressure (10 and 20 mN/m) also positive excess area was observed at gA/DMPC molar ratio between 0 - 0.2 molar fraction of gA. It suggests phase separation in a binary monolayer. The separated phases may not mean pure components, this may suggest, e.g., small 2D clusters of individual gA molecules being attached with a certain number of DMPC molecules in the prevailing pure DMPC phase.

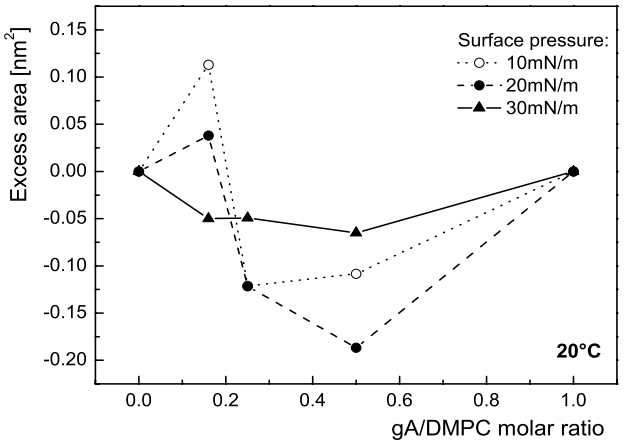

Fig. (3). Plot of the excess area as a function of molar ratio of gA/DMPC at temperature 20 ^{0}C, and at different levels of surface pressures (see inserts at figures).

The molecular interactions in a two-component monolayer can be evaluated by a more detailed examination of the thermodynamics of the system. Such an analysis originated from [6].

The variations of the Gibbs' free energy of a system containing a monolayer is given by

$$\left(\frac{\partial G}{\partial A}\right)_{T,P,n} = \gamma \tag{11}$$

where γ is the surface tension and S is the interfacial area. If we have a mixed monolayer with both components (1 and 2) constrained to remain in the surface, and variations of surface pressure are achieved by moving a barrier, at constant T and P, we have

$$dG = -Ad\pi \tag{12}$$

We define ΔG_m^{π}, Gibbs' free energy of mixing at surface pressure π, by

$$\Delta G_m^{\pi} = G_{12}^{\pi} - x_1 G_1^{\pi} - x_2 G_2^{\pi} \tag{13}$$

The evaluation of the free energy of mixing can be made directly from the π-A curves of the pure and mixed monolayers. It is often useful to consider the excess Gibbs' free energy of mixing, above that found for an ideal mixed film

$$\Delta G = \int_0^{\pi} \left(A_{12} - x_1 A_1 - x_2 A_2\right) \mathrm{d}\pi \tag{14}$$

The value of ΔG provides information whether the particular interaction is energetically favorable ($\Delta G<0$) or not ($\Delta G>0$), while for $\Delta G=0$ ideal mixing takes place. The value of ΔG as a function of gA/DMPC molar ratio for different temperatures is shown in Fig. (**4**). For DMPC molecules in gel state (20°C) we can see negative values of the Gibbs free energy in wide range of molar ratios. This means that the interaction between gA and phospholipids is favorable and that these molecules closely interact with each other forming stable complexes.

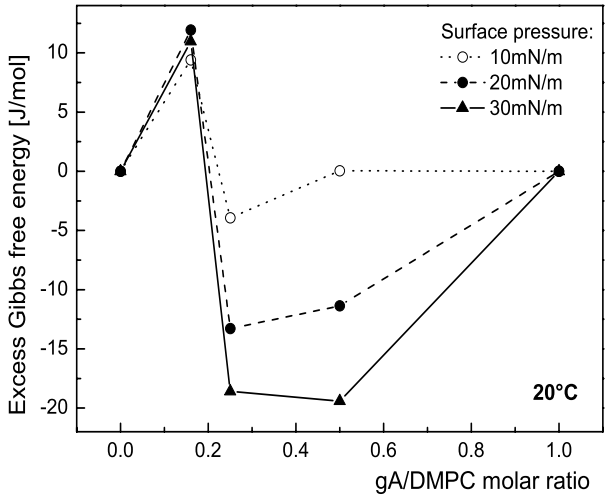

Fig. (4). Excess Gibbs free energy as a function of gA molar ratio for temperature 20 ^{0}C at surface pressures of 10, 20, 30 mN/m (see insets at the figures).

If the temperature variation of the π–A curves and excess Gibbs' free energy temperature dependence can be determined, it is also possible to evaluate the excess entropy and heat contributions (excess enthalpy). As done by [7]:

$$\Delta S = -\left(\frac{\partial \Delta G}{\partial T}\right)_{\pi} - \Delta A\left(\frac{\partial \gamma}{\partial T}\right) \qquad (15)$$

where ΔA refers to the excess area and the derivative of surface tension of pure water with respect to temperature is $\partial\gamma/\partial T$ = –0.154mN/m·K.

The plot of the excess entropy as a function of gA/DMPC molar ratio is shown in Fig. (**5**). It is seen from this figure, that a sharp minimum exists for all surface pressures at gA/DMPC molar ratio 0.25. The excess entropy is almost independent of temperature.

5. DISCUSSION

The primary structure of gramicidin A was first reported in [8] as follows:

N – terminus / formyl-L-Val^1-Gly^2-L-Ala^3-D-Leu^4-L-Ala^5-D-Val^6- L-Val^7- D-Val^8-L-**Trp**^{9}-D-Leu^{10}-L-**Trp**^{11}-D-Leu^{12}-L-**Trp**^{13}-D-Leu^{14}-L-**Trp**^{15}-ethanolamine / C – terminus.

The peptide is composed of hydrophobic residues and is capped at its ends, which prevents it from becoming charged as the pH is altered. In a lipid bilayer environment, which mimics a cell membrane, gramicidin A adopts a helical structure [9]. Due to the alteration of D- and L-amino acid residues, this is a β helix.

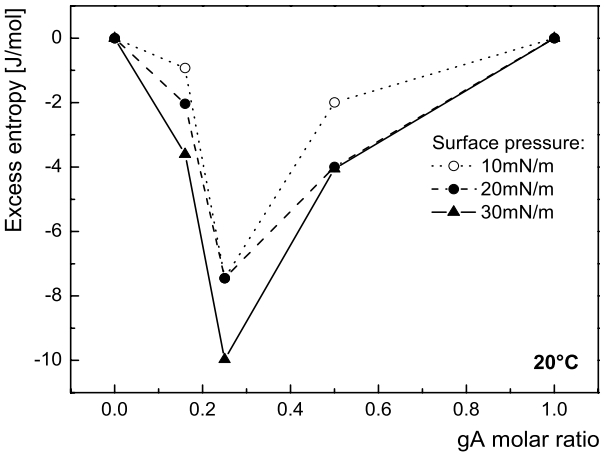

Fig. (5). Plot of the excess entropy of mixing as a function of gA molar ratio for T=20 ^{0}C (right view).

Another important feature of gramicidin A is that in the environment of a lipid bilayer, two molecules dimerize in a head-to-head manner, producing a ion channel spanning the membrane 2.6 nm long with a central pore approx. 0.4 nm in a diameter. It is selectively permeable for different monovalent ions. Depending on surrounding solvent as well as in a crystalline form, several alternative various interwined helical dimers were reported.

According to our experimental results, two types of processes are realized during a compression of the monolayer: formation of the gA dimers and a change of the secondary structure of gA in the region of Trp C-terminus caused by the interaction with surrounding lipid polar groups. These phenomena are certainly determined by the protein/lipid molar ratio as well as by the thermodynamic state of the monolayer characterized by temperature and surface pressure. Temperature accounts also for the phase state of the lipid molecules (gel state below 24 °C for DMPC and liquid-crystalline state with disordered aliphatic chains caused by gauche rotations and kinks above 24 °C). As it unambiguously comes out from the thermodynamic properties (excess area, Gibbs free energy, excess entropy) the rate of gA-DMPC interaction is maximum at around x_{gA} = 0.25. At this situation the phospholipid molecules *via* their carbonyl moieties dominantly interact with the single helical gA, which mostly stands upright on the surface being anchored by its C-terminus to water, and prevent a formation of the intertwined helical gA dimers.

In the mixtures with a higher gA content (molar ratios 0.5, 1.0) the effect of surrounding DMPC molecules is weaker and during the course of monolayer compression, at approx. 2.5 – 3.0 nm^2 per molecule, the plateau region in the isotherms in Fig. (**2**), the dimer formation is progressing. This corresponds with negative values in the excess areas (Fig. **3**) at surface pressures higher than 10 mN/m for the gA molar ratio of 0.3 and more, when the protein helices are more effectively spaced in the intertwined manner.

The situation is rather different at the molar ratios gA less than 0.3 - 0.4. In the excess area dependences the positive deviations dominate, gA retains its single-strained configuration. Four Trp residues interact *via* the N-H hydrogen bonds with the phospholipid head groups with a maximum

effect at a molar ratio of 0.25, i.e. four lipid head groups are in contact with four Trp groups involved in a helix. This leads to a conformational change in the region close the C – terminus and to a change in the charge density distribution on the groups being affected by hydrogen bonding.

CONCLUSIONS

The dominant processes in the gA-DMPC, associated with molecular ordering and molecular conformation caused by lipid – protein interaction and by the change in electrical properties, take place in the monolayer at the air/water interface in the thermodynamic states typical of gaseous 2D phase.

The decisive factor is the interaction of the polar regions in lipids with the polar indole moieties, this results in the fixation C-termini of gA to polar surface of each lipid monolayer constituting the bilayer membrane and hence for creation of a helical dimer which functions as an ion channel through a membrane.

In perspective, the channel-like structures should provide the ability to design and build systems that selectively control the flow of virtually any ion. Applications ranging from new kidney dialysis materials to novel wastewater treatment methods are possible.

ACKNOWLEDGEMENTS

The work was supported by the Agency for Promotion of Research and Development, projects No. APVV-0362-07 and APVV-0290-06. This publication originated from the project "National centre for research and applications of renewable energy sources" (IMTS 26240120016) of Operational programme Research and development financed by European fund of regional advancement.

REFERENCES

[1] Vitovič, P.; Weis, M.; Tomčík, P.; Cirák, J.; Hianik T.; Maxwell displacement current allows to study structural changes of gramicidin A in monolayers at the air-water interface. *Bioelectrochemistry*, **2007**, *70*, 469-480.

[2] Lavoie, H.; Blaudez, D.; Vaknin, D.; Desbat, B.; Ocko, B.M.; Salesse, Ch.; Spectroscopic and structural properties of valine gramicidin A in monolayers at the air-water interface. *Biophys. J.*, **2002**, *83*, 3558-3569.

[3] Kaganer, V.M.; Möhwald, H.; Dutta, P; Structure and phase transition in Langmuir monolayers. *Rev. Mod. Phys*., **1999**, *71*, 779-819.

[4] Maget-Dana, R.; The monolayer technique: a potent tool for studying the interfacial properties of antimicrobial and membrane-lytic peptides and their interactions with lipid membranes. *Biochim. Biophys. Acta*, **1999**, *1462*, 109-140.

[5] Dynarowicz-Łątka, P.; Dhanabalan, A.; Oliveira, O. N. Jr.; Modern physicochemical research on Langmuir monolayers. *Adv. Colloid Interface Sci.*, **2001**, *91*, 221-293.

[6] Goodrich, F. C.; In Proceedings of the 2nd International Congress on Surface Activity Vol I, London, England, 1957; Schulman, J.H., Ed., **1957**, p. 85.

[7] Bacon, K.J.; Barnes, G.T.; Two-component monolayers, IV. The excess enthalpies and entropies of mixing in the octadecanoIdocosyl sulfate system. *J. Colloid Interface Sci.*, **1978**, *67*, 70-77.

[8] Sarges, R.; Witkop, B.; The structure of valine- and isoleucine-gramicidin A. *J. Am. Chem. Soc.*, **1965**, *87*, 2011-2020.

[9] Wallace, B. A.; Recent advances in the high resolution structures of bacterial channels: Gramicidin A. *J. Struct. Biol.*, **1998**, *121*, 123-141.

Received: August 20, 2009 Revised: September 14, 2009 Accepted: December 02, 2009