# Study of Gramicidin A−Phospholipid Interactions in Langmuir Monolayers: Analysis of Their Mechanical, Thermodynamical, and Electrical Properties

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The mechanisms of interactions between gramicidin A (gA) and dimyristoylphosphatidylcholine (DMPC) in monolayers formed at the air−water interface were studied by analyzing their mechanical, thermodynamical, and electrical properties evaluated from measurements of pressure−area isotherms and of Maxwell displacement currents (MDC). A contactless method of recording MDC enabled us to monitor changes in the charge state of the monolayer-constituting molecules and to find the relation between a phase state of the monolayer and structural transitions of gA. The peptide−lipid interactions were quantified in terms of the excess of Gibbs free energy, excess entropy, as well as the molecular dipole moments at various gA/DMPC molar ratios, at various temperatures (in the gel phase and also in the liquid-crystalline phase of DMPC molecule), and at various surface pressures. It was found that the strongest interactions between gA and DMPC took place at the gA/DMPC molar ratio at around 0.25. At this monolayer composition, the phospholipids, via their carbonyl moieties, dominantly interact with the single helical gA, which mostly stands upright on the surface and is anchored by its C-terminus to the water surface, and prevent the formation of the intertwined helical gA dimers. The optimum ratio was confirmed also by anomalous electrical behavior of electrical dipole moments derived from MDC measurements.

## 1. Introduction

Monomolecular, insoluble layers on the surface of a liquid, termed the Langmuir monolayers, are formed as a result of a self-assembled process. Organic molecules constituting the monolayers are amphiphilic, and they spontaneously spread at the air/water interface. The Langmuir monolayer is a very suitable model for the study of self-assembly processes in two dimensions. The water phase provides an ideally planar and smooth surface as a substrate. A pair of thermodynamic quantities—temperature and surface pressure—can be easily controlled. Surface pressure can be changed by moving a barrier over the surface. Such mechanical compression, which is analogous to hydrostatic pressure in 3D systems, is not available in other 2D systems and arrangements. In addition, the interaction between the molecules in the monolayer can be systematically affected by chemical modification of their polar and nonpolar parts (e.g., the chain length can be varied by subnanometer steps) or by changes of pH or ionic strength and composition of the water subphase. The Langmuir monolayers are also a convenient model of biomembranes because they can be considered as two superimposed lipid monolayers.

Biological membranes represent a basic structural component of all biological systems. They participate in the transport of ions and other molecules and are involved in the metabolic processes and signal transduction. The lipid monolayer, as one of the simplest models of biomembranes,[1,2] exhibits promising applications for studying various processes connected with functioning biomembranes, including the mechanisms of lipid−protein interactions at the molecular level.[3−5] Among integral proteins, the ion channels are of special interest. They behave like an ion-selective gate with switching sensitive to external conditions. Considering a different conformation between the open and closed state of the ionic channel, one can expect the effect of the channels on the physical properties of biomembranes or model membranes and vice versa. Gramicidin, secreted by *Bacillus brevis*, is considered as a model peptide for studies of mechanisms of ion transport as well as the mechanisms of protein−lipid interactions in biomembranes. Four different known types of gramicidin differ in their amino acid composition and structure. Gramicidin S is cyclic decapeptide, whereas gramicidins A, B, and C are helical pentadecapeptides. The gramicidins A, B, and C differ in the amino acid residue at the 11th position. Gramicidin A has a tryptophan, B has a phenylalanine, and C has a tyrosine at this site. The primary structure of gramicidin A was first reported in 1965 and is as follows:[6]

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 when the pH is altered. Depending on the surrounding solvent, as well as when in a crystalline form, several different

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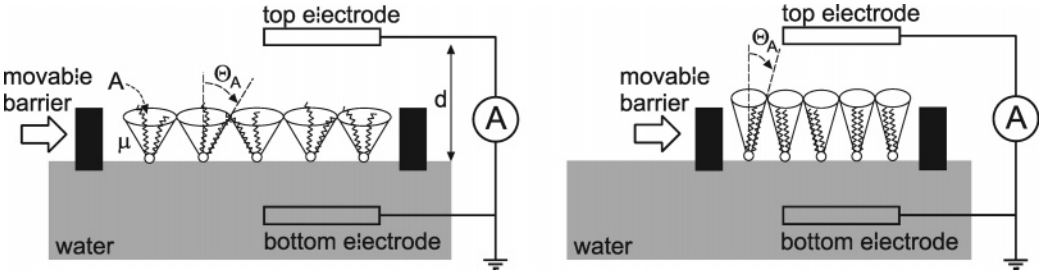


**Figure 1.** Schematic view of the experimental setup for the displacement current measurement. Rod-like polar molecules execute precessional motion on the air/water interface with the maximum tilt angle $\Theta_A$ ($A$ and $\mu$ are the area per molecule and the dipole moment of the molecule, respectively). Electrical shielding of the top electrode is not shown.

intertwined helical dimers were reported. These double helical dimers were found also in vesicles of polyunsaturated lipids as well as in planar bilayer lipid membranes under certain conditions.[7] Among the various secondary structures, the double-strand intertwined $\beta^{5,6}$ helix is of special interest. It has a similar length (2.6−3.0 nm) to that of the gA channel in the $\beta^{3,6}$ conformation, which is typical for the gA dimer in lipid bilayers, and thus represents a structural alternative of the gA channel. It is characterized by a different selectivity for various monovalent ions. In perspective, the channel-like structures should provide the ability to design and build systems that control the flow of virtually any ion. Applications ranging from new kidney dialysis materials to novel wastewater treatment methods are possible. The impetus to study gramicidin monolayers is not only biological, but it is important also for the development of biomaterials and biosensors. In this case, pure gramicidin films or films with high ratios of gramicidin to lipid have been employed.

It was shown[8−10] that surface pressure and surface potential measurements of mixed monolayers of various phospholipid−protein combinations are very effective for the investigation of the mechanisms of molecular interaction.

Various experimental techniques have been designed for the evaluation of structural properties and the study of the order parameter in organic thin films situated on the air/water interface.[11,12] However, the measurement of the orientational parameter in the time domain is possible only in a few of them. For the detection of changes in the charge state of a molecule as well as that in their relation to structural and conformational changes, a contactless method was developed based on the analysis of the Maxwell displacement currents (MDC). This method was originally introduced by Iwamoto et al.[13] and subsequently innovated by other authors.[14,15] Capability of the MDC experiment for the investigation of biological membranes was presented in our previous works, including the study of the physical properties of gramicidin−phospholipid monolayers.[16]

In this paper, we report on the analysis of physical properties of the mixed gA−phospholipid monolayers using the results of measurements of surface pressure and MDC. We describe aspects of monolayer technology by focusing on some particular properties of the binary system, such as the mechanical and thermodynamical properties in a monomolecular layer as well as electrical properties (namely, the molecular dipole moment). In each part, methodological importance and its specificity for studying molecular interactions is stressed.

## 2. Materials and Methods

**2.1. Chemicals.** The monolayers were formed from dimyristoylphosphatidylcholine (DMPC) (Avanti Polar Lipids, USA), gramicidin A (gA) (Sigma-Aldrich, USA), or from their mixtures. DMPC, gA, or their mixtures were dissolved in chloroform in a concentration of 1 mg/mL, and a small amount of these solutions (approximately 25 $\mu$L) was spread on the water subphase of the Langmuir trough using a microsyringe (Hamilton, USA). Millipore water (15 MΩ·cm, ELIX 5, Millipore, USA) was used as a subphase. The subphase was thermostated to a temperature of 17 °C (in the MDC experiments) and to 20, 24, and 28 °C (in the analysis of mechanical and thermodynamic parameters) using a LAUDA E200 thermostat (Koenigshofen, Germany) with an accuracy of 0.05 °C.

**2.2. Experimental Methods.** The basic components of the Maxwell displacement current experimental setup attached to the computer-controlled Langmuir trough (model 611, Nima Technology, UK) are schematically shown in Figure 1. The top electrode was situated parallel to the interface, in air, without direct (mechanical or electrical) contact with a floating monolayer. The distance between the top electrode and the water surface was controlled by measuring the electrical capacitance. The displacement current was measured by a Keithley 617 electrometer (Keithley Instruments, Cleveland, Ohio, USA).

The total working area of the trough was 600 cm$^2$, and the compression rate was 50 cm$^2$/min, which corresponds to 0.17 Å$^2$/s per one molecule. The area of the top electrode was $A_E$ = 20 cm$^2$. The monolayer was allowed to equilibrate and the solvent to evaporate for 15 min. This time was found to be sufficient for evaporation of chloroform and stabilization of the monolayer. The surface pressure−area isotherms were measured by the Willhelmy plate method with an accuracy of 0.05 mN/m.

## 3. Experimental Results

Surface pressure−area isotherms, recorded at various temperatures as well as at various gA/DMPC molar ratios, are shown in Figure 2. Pressure−area isotherms have a typical shape as reported earlier for pure DMPC, gA, or their mixtures.[16] We can see that monolayers for pure DMPC are in a liquid-expanded state (LE) at the high area per molecule ($A$ > 0.78 nm$^2$), and at the surface pressure $\pi \approx 8$ mN/m, it turns into the liquid-condensed state (LC). In a gel state of DMPC ($T$ = 20 °C), a plateau is observed at $\pi \approx 25$ mN/m. This plateau is less expressed at $T$ = 24 °C, which corresponds to a phase transition of DMPC, 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 data.[16−18] The shape of the isotherms is not significantly sensitive to temperature in the range of $T$ = 20−28 °C and is characterized by a typical plateau at $\pi \approx 14$ mN/m. The data obtained by PM-IRRAS and X-ray reflectivity methods allowed us to connect the shape of the $\pi$−$A$ isotherms with the structural changes of gA during compression.[18] In particular, the increase in the surface pressure between 0 and 10 mN/m was attributed to formation of an intertwined

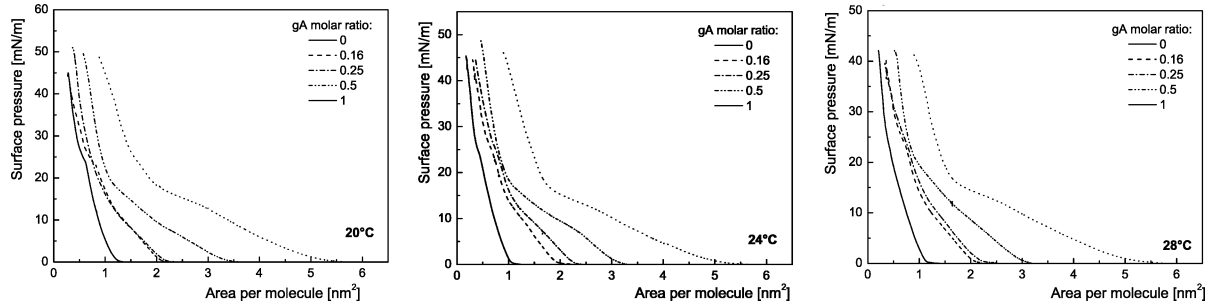


**Figure 2.** Pressure−area compression isotherms of gA/DMPC mixed monolayers and pure components at an air−water interface. Curves correspond to various molar fractions of gA (see insets in the figures): 0 means pure DMPC, and 1 is pure gA. The isotherms were recorded at (A) 20 °C, (B) 24 °C, and (C) 28 °C.

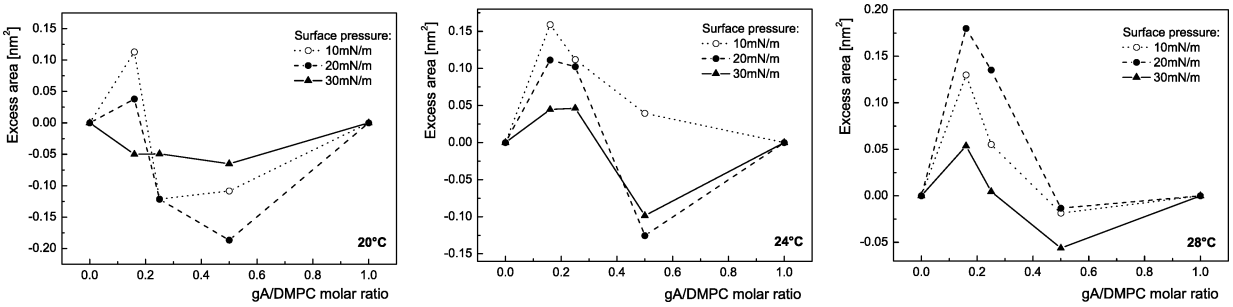


**Figure 3.** Plot of the excess area as a function of the molar ratio of gA/DMPC at various temperatures and at different levels of surface pressures (see insets in the figures).

$\beta$-helix from a disordered secondary structure. From Figure 2, it is also seen that, with an increasing molar ratio of gA, the average area per lipid molecule increases.

Information about the mutual miscibility of the components of a binary system, which is represented by a mixed monolayer, may be obtained from the change in surface area as a function of the molar ratio at constant thermodynamic quantities, temperature and surface pressure. If two components are immiscible or if they behave like an ideal mixture, the excess area of the mixture, $\Delta A$, is defined as

$$\Delta A = A_{12} - x_1 A_1 - x_2 A_2 \tag{1}$$

and is equal to zero. $A_{12}$ is the molecular area in the mixed monolayer at temperature $T$ and surface pressure $\pi$, whereas $A_1$ and $A_2$ are molecular areas in the pure 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/gA molar ratio dependences are shown in Figure 3 for DMPC monolayers in a gel state (20 °C), in the region of a phase transition (24 °C), and in a liquid-crystalline state (28 °C). It is seen from the figure that the excess area isotherms depend not only on the temperature but also on surface pressure. For a low-temperature experiment (gel state of DMPC), a negative excess area for all molar ratios of the gA/DMPC mixtures was observed at a constant surface pressure of 30 mN/m. This suggests a high miscibility of gA and DMPC molecules without creation of domains of pure components. However, at lower surface pressure, positive excess area was also observed at gA/DMPC molar ratios between 0 and 0.2. In the liquid-crystalline state of DMPC, there is positive deviation from linearity in the molar fraction range of 0−0.25 for gA at $\pi = 30$ mN/m and a negative deviation at higher molar fractions. This suggests a phase separation of individual components in a mixed monolayer at a molar fraction of 0−0.25 of gA. Excess area−molar ratio dependence in the phase transition temperature area exhibits complex behavior. For example, in the case of a higher surface pressure ($\pi = 30$ mN/m), the region of cluster formation is extended up to 0.3 gA/DMPC molar ratio. However, for a lower surface pressure ($\pi = 10$ mN/m), the clusters are formed in all molar fractions of gA/DMPC. Similar behavior of the gA−DMPC mixed monolayers was reported earlier[16] and also for the gel state of gA−DPPC mixed monolayers.[17] However, in contrast with ref 17, we did not observe positive deviation from the excess area in a gel state of DMPC. These differences between DMPC and DPPC monolayers may be due to a different mismatch between gA−DMPC and gA−DPPC due to different lengths of the hydrophobic chains of these two phospholipids.

**3.1. Mechanical Properties.** The measurement of the collapse pressure is a very sensitive tool for the study of monolayer stability. The analysis is based on the phase rule[19] introduced by Crisp. If temperature and surface pressure are constant, the number of degrees of freedom $F$ is defined as

$$F = C^{\mathrm{B}} + C^{\mathrm{S}} - P^{\mathrm{B}} - (q - 1) \tag{2}$$

where $C^{\mathrm{B}}$ is the number of components in the volume, $C^{\mathrm{S}}$ is the number of components on the surface, $P^{\mathrm{B}}$ the number of phases in the volume, and $q$ is the number of monolayer phases that are in equilibrium with each other. In our case, $C^{\mathrm{B}} = 2$ (air and water), $C^{\mathrm{S}} = 2$ (DMPC and gramicidin A), and $P^{\mathrm{B}} = 2$ (gas and liquid). After the substitution, eq 1 is reduced to

$$F = 3 - q \tag{3}$$

We will use the phase rule in the region of critical collapse, but it is also applicable for 2D phase transitions (L−S or LE−LC transitions). If the two components are miscible, then two phases (e.g., S and the collapsed phase) are in equilibrium. Therefore $q = 2$, and the system still has one degree of freedom. If the two components are immiscible, then $q = 3$, and the system has no degrees of freedom. However, in this case, the surface pressure is independent of the monolayer composition.[20]

Membrane curving due to thermal fluctuations is essential for the shape and/or the conformations of the membranes, as well as for cracks and defect generation. The elastic modulus

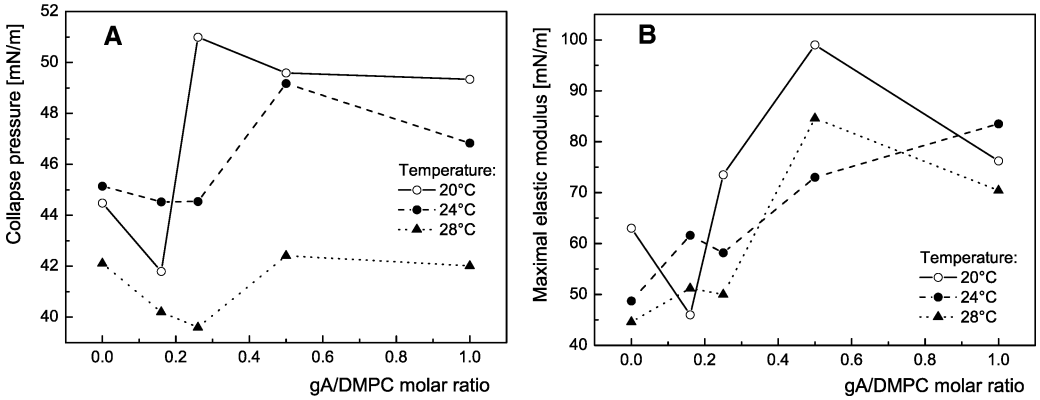


**Figure 4.** Collapse pressure (A) and maximum value of elastic modulus (B) as functions of the gA/DMPC molar ratio for different temperatures: ○, 20 °C; ●, 24 °C; ▲, 28 °C.

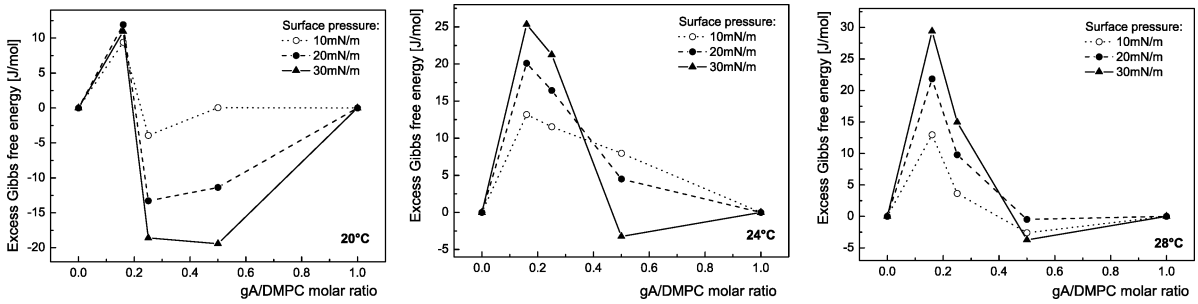


**Figure 5.** Excess Gibbs free energy as a function of the gA molar ratio for three different temperatures and surface pressures (see insets in the figures).

characterizes the elasticity of the monolayer and, in analogy with bulk materials, is given by

$$|E| = -A\left(\frac{\partial \pi}{\partial A}\right)_T \tag{4}$$

where $\pi$ is the surface pressure, $A$ is the area per molecule, and $T$ is temperature. The elastic modulus reflects the degree of compressibility of the monolayer.

Figure 4 shows the plot of the collapse pressure and maximum values of the elasticity modulus of monolayers as a function of the gA/DMPC molar ratio. The collapse pressure reflects the degree of the stability of the monolayers. More stable monolayers are characterized by a higher collapse pressure. We can see that, with the exception of the low molar ratio region of gA/DMPC (0−0.2), both the collapse pressure and the elasticity modulus increase with an increase in the content of gA in mixed monolayers. This result is in good agreement with those obtained by analysis of the excess area of the monolayer, which suggests formation of stable gA−DMPC complexes.

**3.2. Thermodynamic Properties.** The stability of the mixed monolayer can be also determined by evaluating the excess Gibbs free energy of the mixture following the Goodrich method,[21] performing the integration of the surface pressure−area isotherm up to the selected surface pressure $\pi$

$$\Delta G = \int_0^{*\pi} (A_{12} - x_1 A_1 - x_2 A_2)\mathrm{d}\pi \tag{5}$$

The value of $\Delta G$ 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 $\Delta G$ as a function of the gA/DMPC molar ratio for different temperatures is shown in Figure 5. For DMPC molecules in the gel state (20 °C), we can see negative values of the Gibbs free energy over a wide range of molar ratios. This means that the interaction between gA and the phospholipids is favorable and that these molecules closely interact with each other, forming stable complexes. The liquid-crystalline state of DMPC (28 °C) is characterized by a positive $\Delta G$ in the range of $x_{gA} = 0-0.5$. Thus, in this case, the interaction between gA and DMPC is not favorable, and phase separation takes place in a monolayer. These results correspond to the excess area−molar ratio analysis (Figure 3). The mixed monolayer close to the phase transition shows thermodynamic properties similar to those of a liquid-crystalline state.

The excess entropy of a mixed monolayer can be calculated from a temperature dependence of the excess Gibbs free energy (Figure 6 A), the excess area per molecule, and the derivative of the surface pressure of pure water with respect to temperature ($\partial\gamma/\partial T = -0.154$ mN/m·K), as done by Bacon and Barnes[22]

$$\Delta S = -\left(\frac{\partial \Delta G}{\partial T}\right)_\pi - \Delta A\left(\frac{\partial \gamma}{\partial T}\right) \tag{6}$$

The excess enthalpy of the mixture can be calculated using the excess Gibbs energy and entropy

$$\Delta H = \Delta G + T\Delta S \tag{7}$$

Figure 6A shows the excess Gibbs free energy as a function of temperature for selected gA molar ratios. The deviation from linearity at higher temperatures is caused by the phase transition in a monolayer. Therefore, we considered only the first two points. The plot of the excess entropy as a function of the gA/DMPC molar ratio is shown in Figure 6B. It is seen from this figure that a sharp minimum exists for all surface pressures at a gA/DMPC molar ratio of 0.25. The excess entropy is almost independent of temperature.

**3.3. Electrical Properties of Mixed gA−DMPC Monolayers.** The analysis of the results of the measurement of MDC is based on the assumption that each molecule behaves like a weak

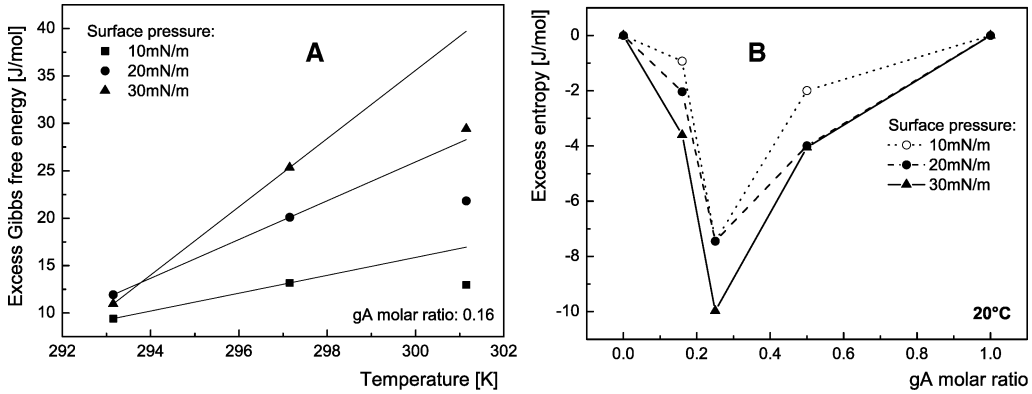


**Figure 6.** (A) The plot of the excess Gibbs free energy as a function of temperature for the gA molar ratio $x_{gA} = 0.16$. (B) The plot of the excess entropy of mixing as a function of the gA molar ratio for $T = 20$ °C.

dipole. Individual molecules have random orientation within a certain angle and execute a random precessional motion with a maximal possible tilt $\Theta_A$ from the vertical axis. In general, we could consider the molecule as a rod-like rigid body without the possibility of bending.

If we consider the organic film as a system of electric dipole moments, it is possible to calculate the induced charge on the upper electrode by the method of images as follows

$$Q_i = \langle \mu_z \rangle NG = \mu \langle \cos \Theta \rangle NG \tag{8}$$

where $\mu$ is the dipole moment of one molecule ($\mu_z$ is a projection of $\mu$ to the normal), $N$ is the number of molecules under the top electrode, and $G$ is the geometric factor depending only on the distance between the top electrode and the top plane of the monolayer and on the shape and the area of the upper electrode. The $\langle \cos \Theta \rangle$ is the statistical mean value of cos $\Theta$, where $\Theta$ is the angle between the vector of the dipole moment and the normal. Detailed analysis of the dipole moment projection of simple fatty acids is described elsewhere.[23]

The current flowing in the outer circuit can be expressed as changes in the induced charge in time

$$I = \frac{\partial Q_i}{\partial t} = \mu NG \frac{\partial \langle \cos \Theta \rangle}{\partial t} + \mu \langle \cos \Theta \rangle G \frac{\partial N}{\partial t} \tag{9}$$

By integrating the displacement current with respect to the time, the induced charge, $Q$, can be obtained, and in this way, we also evaluated the vertical component of the molecular dipole moment. Thus, the dipole moment projection to the normal should be calculated as

$$\mu_z = \mu \langle \cos \Theta \rangle = \frac{1}{GN} \int I \mathrm{d}t \tag{10}$$

The plots of surface pressure, MDC, as well as the calculated dipole moment projection versus the area isotherm are shown in Figure 7. As we can see, the phase transitions visible from the surface pressure versus area isotherm correspond to calculated dipole moment projections to the normal for pure components. During the compression of the monolayers, a reorientation of the molecules takes place. From Figure 7, the relation between the changes in the phase transition and the reorientation of the molecules, which resulted in changes of MDC and the dipole moments, can be seen. For a monolayer of pure gramicidin A, the reorientation of molecules took place already in the gaseous phase. This is suggested by a peak in a MDC−area plot at 7.5 nm$^2$ per molecule and by a sharp increase in the dipole moment projection. Also, for pure DMPC,

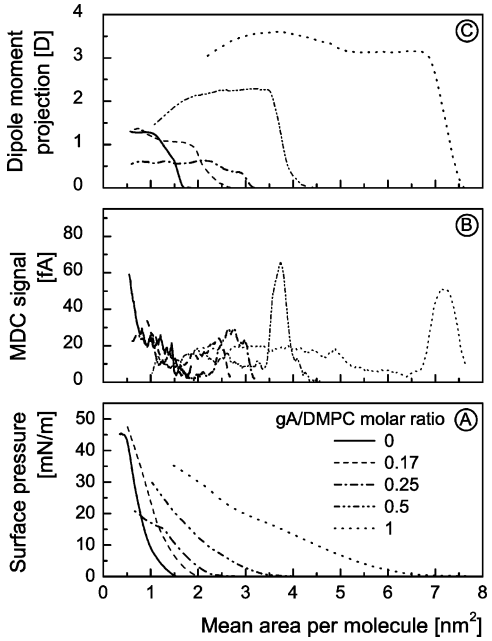


**Figure 7.** Surface pressure (A), Maxwell displacement current (B), and dipole moment projection to the normal (C) versus the mean area per molecule for various gA/DMPC molar ratios.

monolayer dipole moment projections properly represent phases observed in the surface pressure−area isotherm. Dipole moment projections of gA−DMPC mixed monolayers indicate average dipole moment projections of a two-component system. The maximum value of the dipole moment projection of DMPC and gA was $\mu_{DMPC} = 1.28$ D and $\mu_{gA} = 3.60$ D. If the components of a mixed monolayer are immiscible, the maximum average value of the dipole moment of the mixture is given by a superposition of the dipole moments. Thus, the excess dipole moment of a mixture, $\Delta\mu$, is given by

$$\Delta\mu = \mu_{12} - x_1\mu_1 - x_2\mu_2 \tag{11}$$

where $\mu_1$ and $\mu_2$ represent the maximum value of dipole moments of pure components, and $\mu_{12}$ is the maximum value of the dipole moment of the mixture for a particular molar ratio of the components. The maximum value of the dipole moment projection and the excess dipole moment of the mixed monolayers as functions of a gA/DMPC molar ratio are presented in Figure 8. We can see the development of a maximal dipole moment of gA−DMPC mixed monolayers as a function of the gA molar ratio. The deviation from linearity for all mixtures is noticeable. This fact is confirmed by an excess in the dipole

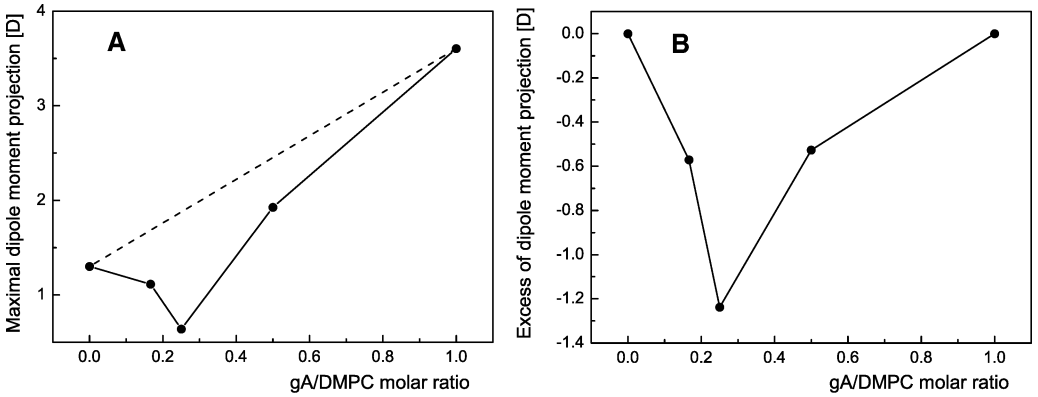


**Figure 8.** (A) Maximal value of the dipole moment projection and (B) the excess of maximum value of the dipole moment projection as a function of the gA/DMPC molar ratio.

moment projection versus molar fraction plot (Figure 8B). The sharp minimum is reached for a gA/DMPC molar ratio of 0.25.

## 4. Discussion

The miscibility of two compounds in a mixed monolayer at the water−air interface can be deduced as a result of the behavior of the molecular areas as a function of the mixture composition at constant surface pressure. Positive or negative deviation from an ideal dependence of the excess area as a function of the gA/DMPC ratio characterizes repulsive or attractive mutual interactions between the monolayer components (gramicidin A and phospholipid molecules). As it is obvious from Figure 3, the stable state represented by attractive interactions is well expressed for a gel state of a monolayer at a gA molar ratio of $x_{gA} = 0.25-0.5$ and disappears at higher temperatures.

Mechanical properties of the Langmuir film can be evaluated in terms of the elastic modulus and collapse pressure. The elastic modulus of the monolayers, also the so-called surface compressibility modulus, characterizes the elasticity of the film. High rigidity (a high value of the elastic modulus) is usually induced by strong attractive forces between molecules or mixture components. In Figure 4B, the maximum values of the elastic modulus of the mixed monolayers are drawn as a function of the gA/DMPC molar ratio. The maximal value of the elasticity modulus is reached at a gA/DMPC molar ratio of 0.5. The analysis, based on phase rules using the determination of the collapse pressure as a function of the monolayer composition, demonstrates the existence of the most stable structure for gA/DMPC molar ratios in a range of 0.25−0.5. With increased temperature, both the elastic modulus and the collapse pressure decrease. This suggests the decrease of miscibility of the monolayer components.

From analysis of thermodynamic properties of the monolayers, one can obtain more information about the energy of the stable state of a mixed monolayer. The behavior of $\Delta G$ (see Figure 5) is analogous to that observed for the excess area. In fact, the negative values of $\Delta G$ are observed for a gA/DMPC molar ratio in a range of 0.25−0.5 and for low temperatures. The dependence of excess entropy (Figure 6) on the gA/DMPC molar ratio shows a high formation of an ordered structure for gA at a molar ratio of 0.25. The excess entropy function practically did not depend on temperature. The entropy contribution is also a principal component of the excess enthalpy, $\Delta H$, for mixed monolayers. The excess enthalpy at an optimum gA/DMPC molar ratio ($x_{gA} = 0.25$) exhibits an energy minimum, which is close to the thermal energy value at 300 K. Therefore, the ordered and stable structure can be preserved only at lower temperatures.

Study of the electrical properties of the monolayers using the Maxwell displacement current method allows us to determine the values of the dipole moment projection as well as the analysis of the excess dipole moment as a function of the gA/DMPC molar ratio. The MDC technique allowed us to determine the projection of the dipole moments of DMPC and gA to a direction normal to a monolayer and resulted in maximum values of $\mu_{DMPC} = 1.28$ D and $\mu_{gA} = 3.60$ D. The analysis of the results from the determination of the excess dipole moment revealed a noticeable negative value at a gA/DMPC molar ratio of $x_{gA} = 0.25$. This indicates a change in the conformational charge state of the monolayer components due to a strong mutual interaction between molecules. This observation is in good agreement with thermodynamic properties as well as with mechanical properties of the mixed monolayer.

In the amino acid chain of gramicidin A, four Trp residues lie near the C-terminus of the molecule and are in direct contact with the head group region of the membrane. The orientation of these residues is believed to play a crucial role in the formation of the channel structure and in cation transfer.[24] Trp residues, which contain a polar indole moiety, are known to mediate interactions between the hydrophobic domain of adjacent lipid molecules and cations inside the ion channel.[25] The existence of two different hydrogen-bonding motifs for Trp residues was demonstrated in previous studies.[26,27] The N−H on Trp forms a hydrogen bond with surrounding water molecules in one case and with the carbonyl moieties on the phospholipids in the other case.

Alteration of L- and D-amino acid residues in the gA molecule facilitates the formation of at least two different dimer conformations, a double helix and a helical dimer.[6,24] The helical dimer, which is considered by many investigators as an ion channel in lipid bilayers, contains two monomers, connected head-to-head through their N-termini, in the middle of the membrane. On the other hand, the double helix is formed from two intertwined monomers. The latter structure has been observed in X-ray crystal structure studies. However, it has been shown by FTIR experiments that the double helix of gA appears in Langmuir monolayers of pure gramicidin A at the air/water interface upon compression.[17] Alternatively, the dimer configuration is probably preferable in the presence of lipids.[28]

The superior advantage of the Maxwell displacement current technique lies in the fact that it enables the detection of phenomena, which occur in a monolayer even at negligible values of surface pressure. According to the experimental results, two types of processes are realized during compression of the

gA−lipid monolayer: formation of the gA dimers and a change of the secondary structure of gA in the region of the Trp C-terminus caused by the interaction with surrounding lipid polar groups. These phenomena are certainly determined by the peptide/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 (the gel state below 24 °C for DMPC and the liquid-crystalline state with disordered aliphatic chains above 24 °C). As it univocally comes out from both the mechanical and thermodynamical properties (maximum elastic modulus, excess area, Gibbs free energy, and excess entropy), the gA−DMPC interaction is strongest at a gA/DMPC molar ratio of 0.25. At this ratio, the phospholipid molecules dominantly interact via their carbonyl moieties with the single helical gA, which mostly stands upright on the surface and is anchored by its C-terminus to the water, and prevent the formation of the intertwined helical gA dimers.

In the mixed monolayers with a higher gA content (molar ratios of 0.5, 1.0), the effect of surrounding DMPC molecules is weaker. Therefore, during the monolayer compression, at approximately 2.5−3.0 $nm^2$ per molecule, the plateau region in the isotherms (Figure 2) corresponding to the dimer formation is progressing. This is accompanied by negative values of the excess areas (Figure 3) at surface pressures higher than 10 mN/m for the gA molar ratio of 0.3 and higher when the peptide helices are more effectively spaced in the intertwined manner. By intercalating the helices with antiparallel orientation, the vertical component of the molecular dipole moment starts to decrease upon further compression. This fact is documented in Figure 7 for the curves that corresponds to gA/DMPC molar ratios of 0.5 and for pure gA (i.e., a molar ratio of 1). With a decrease of the gA content, the maximum dipole moment diminishes approximately proportionally following the ideal mixing rule.

The situation is rather different at the gA/DMPC molar ratios of less than 0.3−0.4. In the plot of the excess area−molar ratio (Figure 3), the positive deviations dominate; gA retains its single-stranded 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., the lipid head groups are in contact with four Trp groups involved in a helix). This leads to a conformational change in the region close to the C-terminus and to a change in the charge density distribution on the groups being affected by hydrogen bonding. The resulting maximum dipole moment per a molecule falls down even below the value characteristic of a lipid (i.e., 1.28 D). However, this value remains unchanged upon further compression.

The dominant processes associated with molecular ordering and molecular conformation caused by lipid−protein interactions and by the change in electrical properties take place in the mixed gA−DMPC monolayers in the thermodynamic states typical for the 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 of the C-termini of gA to the polar surface of each lipid monolayer constituting the bilayer membrane and hence in the creation of a helical dimer, which functions as an ion channel in a membrane.

This effect is also observed in the dipole moment−area isotherms. The fall of the dipole moment projection of gA is induced by the dimer formation. However, the dipole moment projection of the gA−DMPC mixture is not only the superposition of the signals from individual components, but it also characterizes the mutual lipid−protein binding. Hence, it is possible to quantify the interactions by means of the excess dipole moment.

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