Gramicidin A Channel in a Matrix from a Semifluorinated Surfactant Monolayer

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Gramicidin A, a polypeptide antibiotic forming transmembrane ion channels, has been incorporated into a Langmuir monolayer formed by a semifluorinated alkane (SFA). In this work, partially fluorinated tetracosane, perfluorohexyloctadecane (F6H18), has been applied, aiming at finding a suitable matrix for gramicidin A to be transferred onto solid support for a biosensor design. For this purpose, the physiological conditions were of special interest (mixed monolayers containing low gramicidin proportion and the surface pressure of 30 mN/m). Mixed monolayers of gramicidin and SFA were found to be miscible within the whole range of mole fractions. A very significant increase of the stability of SFA monolayer has been found in the presence of gramicidin, even at such a low proportion as X(gramicidin) = 0.1, which is reflected in a 3.5-fold increase of the collapse pressure value of mixed monolayer as compared to the film from pure SFA. This interesting phenomenon has been interpreted as being due to the existence of a strong dipole−dipole interaction between both film-forming molecules. Opposite sign of the measured electric surface potential for gramicidin and SFA, resulting from different directions of the dipole moment vectors in both film molecules, implies that the ordered, antiparallel orientation of the dipole moments in the mixed gramicidin/SFA system can be responsible for its extremely high stability.

Introduction

Gramicidins are pentadecapeptides of antimicrobial activity produced by a soil bacterium *Bacillus brevis* during its sporulation phase.[1] They have the following sequence: HCO−Lval−Gly−Lala−DLeu−Lala−Dval−Lval−Dval−LTrp−Dleu−LX−Dleu−LTrp−DLeu−LTrp−$NHCH_2CH_2OH$, where X is Trp, Phe, or Try at the ratio of ca. 7:1:2 (gramicidin A, B, and C, respectively).[2,3] The natural mixture of gramicidins is denoted as gramicidin D, whereas a cyclic form of this antibiotic is named gramicidin S. Gramicidin was isolated for the first time by Dubes and co-workers in 1939, the first microbial antibiotic ever isolated.[4,5] The structure of gramicidin was proposed first by Ramachandran[6] and Sarges[7] and later refined by Veatch.[8] Because this peptide has its N terminus blocked by the rest of formic acid, whereas the C terminus is substituted by aminoethanol, the molecule is uncharged. The alternating sequence of amino acids of opposite chirality is crucial for the peptide's structure, because the adjacent side chains protrude from the same side of the β-sheet, forming a molecular channel. Apart from glycine in position 2, all of the amino acids in this pentadecapeptide possess hydrophobic side chains. Therefore, gramicidin is practically water-insoluble and has an affinity toward lipid bilayers. Moreover, four tryptophan residues in positions 9, 11, 13, and 15 are not only stiff and hydrophobic but also bear a significant permanent dipole moment, which is of utmost importance for establishing interactions with lipids as well as for ion transport abilities through this molecular channel.[9,10] Gramicidin was found to function as an ionic channel selective for monovalent cations, whereas divalent cations, such as Ca^{2+} and Cd^{2+}, block it, being bound at its mouth.[11,12] Because of its simplicity, low molecular mass, and its structure refined to atomic resolutions, gramicidin is probably the best-studied ion channel. It has also been employed for the design and investigation of molecular biosensors.

Most research studies devoted to gramicidin−lipid interactions and to the ion permeability through this channel were performed at the mercury drop electrode[13−18] or in black lipid membranes.[19,20] The experimental approach was complemented with theoretical works (molecular modeling[21] or semimicroscopic studies[22,23]). An alternative to the studies of the gramicidin channel incorporated into lipid bilayers is the investigations of the interactions between this peptide and lipids in floating monolayers at the air/water interface studied with the Langmuir technique. Although denaturation often occurs upon spreading proteins onto the air/water interface, gramicidin A was found to be capable of stable Langmuir monolayer formation.[24] In monolayers, gramicidin seems to maintain its native structure, and at surface pressures above ca. 20 mN/m, the transport of monovalent cations through the molecular channel has been observed.[24,25] In addition to the investigations of pure gramicidin A monolayers, binary mixtures of this peptide with model membrane phospholipids, such as DPPC[3,27,28] or DMPC,[29] were also researched.

Our intention is to design a gramicidin-based biosensor sensitive to monovalent cations, wherein the conducting peptide, gramicidin, is incorporated in the matrix of the supporting monolayer that enables its subsequent transfer onto a solid substrate. According to the review article by Bayley and Cremer,[30] stochastic sensing with protein pores does not have to mimic the molecular devices found in nature. The protein function should be preserved; however, the environment in which the protein is embedded may be artificial, but has to fulfill

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the following requirements: should distribute the peptide homogeneously, and must not modify its structure as well as its functional activity (in the case of gramicidin, the matrix should not interfere with monovalent cation conduction through the channel).

There have already been attempts of finding an appropriate matrix to incorporate gramicidin. Apart from testing natural lipids, ester molecules, which can serve as their models, have been studied in this respect.[31,32] Although these compounds seem to be applicable, they are not chemically stable. Therefore, we have been searching for compounds of a better chemical stability, which can be used instead. We have decided to choose semifluorinated alkanes (SFAs), because they are very stable, chemically inert, and, moreover, can be synthesized at relatively low costs. Their chemical inertness comes from the presence of fluorine atoms and lack of any functional group. These molecules have a diblock structure, in which two moieties, perfluorinated and semifluorinated, are bound covalently. They can be described by the general formula $F(CF_2)_m-(CH_2)_nH$, which is often abbreviated to F*m*H*n*. Despite the lack of any polar group, SFAs are surface active both in solutions of organic liquids[33,34] and at the free water surface, where some of them are known to form stable Langmuir monolayers.[35,36] Langmuir monolayers from SFAs can be transferred onto solid supports, forming highly organized structures.[37−39]

It is worth mentioning that SFAs bear a great dipole moment of ca. 2.8 D. This property is of great importance with regards to their application as a gramicidin-supporting matrix because of expected strong dipole−dipole interactions between SFA molecules and the tryptophan moieties of gramicidin.

In recent years, we have been systematically investigating these interesting molecules both in their pure monolayers as well as in binary mixtures with model surfactants.[40−43] Our results have led us to the conclusion that the most stable monolayers are formed by SFAs possessing both moieties of a considerable length. For instance, a semifluorinated alkane used in our studies with model surfactants was F10H20, perfluorodecyl eicosane, a partially fluorinated triacontane molecule, possessing 30 carbon atoms, the length of which is ca. 3.55 nm.[43] The length of the gramicidin channel was estimated to be ca. 2.5 nm.[1,25] The mismatch of the supporting molecule−peptide channel lengths can exert an unfavorable effect on the function of such a channel as a biosensor,[27] and therefore we have decided to apply shorter SFAs, although their Langmuir monolayers are slightly less stable. We have tested several partially fluorinated tetracosanes (F6H18, F8H16, F10H12, and F12H12), all of them possessing 24 carbon atoms in their molecules and having the length of ca. 3.0 nm, which is comparable to the length of the model phospholipid, DPPC.

In this paper, we show the results obtained only for mixtures of gramicidin A and F6H18, because all of the above-mentioned SFAs have been found to behave in a very similar way. We have applied surface pressure−molecular area ($\pi-A$) isotherms and the dependencies of the electric surface potential change versus molecular area ($\Delta V-A$) upon compression for characteristics of the mixed systems. Our results unambiguously prove that, due to the existence of strong dipole−dipole interactions, semifluorinated alkanes are very good candidates for gramicidin A incorporation.

Experimental Section

Materials. The applied semifluorinated alkanes were synthesized by one of us (M.B.), and the syntheses were described elsewhere.[41,42] Gramicidin A (>90%, <5% H_2O) was purchased from Fluka and used as received. Spectroscopy grade chloroform was supplied by Aldrich.

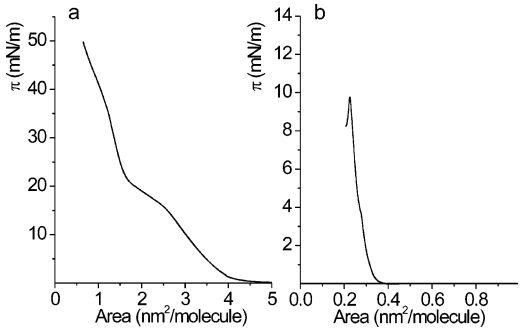

Figure 1. $\pi-A$ isotherms of (a) gramicidin A monolayer, and (b) F6H18 monolayer.

Methods. The spreading solutions for Langmuir experiments were prepared by dissolving each of the investigated compounds in chloroform with a typical concentration of ca. 0.5 mg/mL. Mixed solutions were prepared from the respective stock solutions of both compounds. Ultrapure water (produced by a Nanopure water purification system coupled to a Milli-Q water purification system (resistivity = 18.2 MΩ cm)) was used as a subphase. The subphase temperature was 20 °C, unless other specified, and was controlled to ±0.1 °C by a circulating water system from Julabo. Experiments were carried out with a NIMA 601 trough (Coventry, U.K.) (total area = 600 cm^2), equipped with two symmetrical barriers placed on an anti-vibration table. Surface pressure was measured with the accuracy of ±0.1 mN/m using a Wilhelmy plate made from chromatography paper (Wharman Chr1) as a pressure sensor. After being spread, the monolayers were left for 10 min for the solvent to evaporate, after which compression was initiated with a barrier speed of 15 cm^2/min. Surface potential measurements were performed with the Kelvin probe (model KP2, NFT, Germany) mounted on a NIMA trough. The vibrating plate was located ca. 2 mm above the water surface, while the reference electrode made of platinum foil was placed in the water subphase. The surface potential measurements were reproducible to ±10 mV. Both surface pressure−area and electric surface potential−area isotherms reported here are the averages of at least three experiments.

Results

In Figure 1, the surface pressure−area ($\pi-A$) isotherms of pure gramicidin (Figure 1a) and pure F6H18 (Figure 1b) are shown. The presented isotherms were registered at 20 °C for monolayers spread on the ultrapure water subphase. Regarding the monolayer of pure gramicidin, its $\pi-A$ isotherm is in accordance with the previously published results.[24,25,31] Generally, the surface pressure starts to rise at the molecular area of ca. 5 nm^2/molecule, and the film is in its gaseous state until ca. 4 nm^2/molecule, where a phase transition to the liquid-expanded state occurs. Upon further compression, a pseudo-plateau region appears, which spans over the region of 16−21 mN/m, and is a result of gradual changes in the conformation of the pentadecapeptide from the horizontal to vertical position.[25] The horizontal orientation of the peptide with respect to the free water surface, which occurs in the pressure region below 21 mN/m, prevents the molecule from ion conduction, while at higher surface pressures vertically oriented gramicidin is permeable to monovalent cations.

As far as pure SFA monolayers are concerned, they were a subject of our previous studies.[40−42] Generally for SFAs with

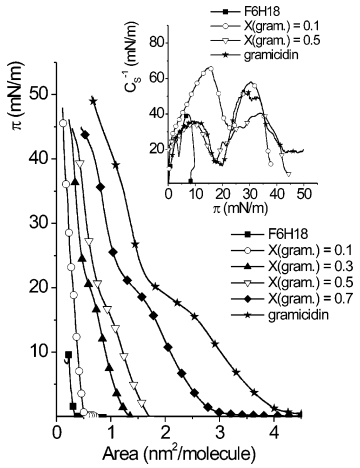

Figure 2. $\pi-A$ isotherms of the investigated mixtures together with compression modulus−surface pressure ($C_S^{-1}-\pi$) dependencies.

15−20 carbon atoms in the hydrogenated part, the lift-off of the $\pi-A$ isotherm is observed at ca. 0.4 nm^2/molecule and the film collapses at ca. 0.28 nm^2/molecule, which is comparable with the cross-sectional area of the perfluorinated chain known from crystallographic data.[44] Low collapse pressure values of SFAs monolayers, ranging from 10 to 15 mN/m (depending on the length and mutual proportion of the fluorinated and hydrogenated moieties of a given molecule), result from the aggregation of film-forming material.

Mixed monolayers of gramicidin A and F6H18 were investigated under the same experimental conditions as pure monolayers discussed above. The following mole fractions of gramicidin, 0, 0.1, 0.3, 0.5, 0.7, 1, were studied. Even at the lowest investigated mole fraction (0.1), gramicidin exerts a profound effect on SFA monolayer; that is, the collapse pressure of ca. 10 mN/m for pure SFA film is shifted to such a high value as 36 mN/m (Figure 2). At this concentration of gramicidin, the shoulder indicating phase transition is hardly visible; however, it starts to become gradually more pronounced upon increase of gramicidin proportion in the mixed monolayer.

To obtain quantitative information about the state of mixed monolayers and to determine exact values of their collapse pressures, compression moduli were calculated. The compression modulus, defined as $C_S^{-1} = -A\,d\pi/dA$,[45,46] is very useful for a precise collapse pressure determination because at the monolayer collapse its value falls to 0, whereas phase transitions are reflected as minima in the $C_S^{-1}-\pi$ graphs. The dependencies of C_S^{-1} versus π for the investigated system are plotted in the inset of Figure 2. For the clarity of presentation, curves for gramicidin mole fractions of 0.3 and 0.7 are not shown in these graphs. The values of C_S^{-1} do not exceed 100 mN/m, which indicates that the monolayer state is liquid-expanded. In the course of the $C_S^{-1}-\pi$ curves, a characteristic minimum can be observed. For pure SFA monolayers, the existence of such a minimum at ca. 5 mN/m was related to the change of the collective molecular tilt angle of the film-forming molecules.[40−42] As it has already been mentioned, the origin of such a minimum on the $C_S^{-1}-\pi$ plots for gramicidin monolayers is due to conformational changes of the peptide upon compression. Interestingly, this minimum at ca. 20 mN/m appears for all of the investigated gramicidin-containing mixtures, including that of the lowest peptide proportion (0.1). Its position is shifted to slightly higher π values upon increasing gramicidin mole fraction.

Simultaneously with $\pi-A$ isotherms, the change of the electric surface potential ΔV was monitored. $\Delta V-A$ isotherms

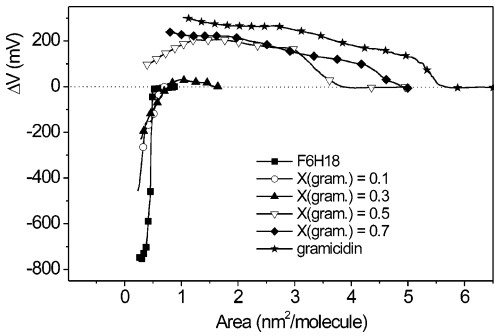

Figure 3. Electric surface potential versus molecular area dependencies ($\Delta V-A$) for the investigated mixed monolayers.

for both pure components and their binary mixtures are gathered in Figure 3. As it can be seen, the sign of ΔV of pure SFA monolayer is negative. The so-called critical area[47] of the electric surface potential change is greater than the lift-off area of the $\pi-A$ isotherm, which is a general tendency observed for the majority of surfactants.[47] The electric properties of pure SFA monolayers were discussed previously.[40−42] The $\Delta V-A$ dependence recorded for pure gramicidin monolayer has a positive sign. It starts to change at ca. 6 nm^2/molecule, that is, at a larger molecular area as compared to the surface pressure rise, and achieves a maximum value of ca. 300 mV in the vicinity of film collapse. Surface potential isotherms of mixed monolayers have an intermediate character. The $\Delta V-A$ dependence for the mixture of gramicidin mole fraction of 0.1 has a negative sign and is qualitatively similar to the $\Delta V-A$ curve of the pure SFA monolayer; however, the value achieved at the film collapse is smaller (ca. −450 vs −750 mV). In the case of X(gramicidin) = 0.3, ΔV has a positive sign and starts to increase at ca. 2 nm^2/molecule, achieving a maximum of ca. 50−100 mV (depending on the mixed system) that begins to decrease upon further compression, reaching a negative value of ca. −100 mV at the film collapse. For a greater gramicidin proportion, the $\Delta V-A$ dependence has a positive sign and is similar to that observed for the pure gramicidin monolayer.

One of the main objectives of this study was to optimize the experimental conditions for the planned deposition of the peptide embedded in the matrix onto solid substrates and the design of new stochastic biosensors made of the gramicidin channel. As it has already been indicated above, for such an application, a small amount of the peptide should be uniformly distributed in a matrix monolayer. Therefore, the mixed monolayer containing the lowest investigated proportion of gramicidin (X(gramicidin = 0.1) can be considered as fulfilling these requirements, and therefore further experiments have been carried out only for this monolayer composition.

However, before the experiments for X(gramicidin) = 0.1 are discussed, some additional experiments performed for pure gramicidin monolayer need to be presented.

The isotherm of pure gramicidin monolayer has already been described at the beginning of the Results. It has been mentioned that at the π values from ca. 16−21 mN/m, gramicidin undergoes a transition from its horizontal to vertical orientation, and in the latter conformation the peptide is active as an ionic channel. Therefore, for a sensor construction, monolayers should be deposited on a solid substrate from a surface pressure region exceeding 21 mN/m. According to the literature,[48] the surface pressure in biological membranes corresponds to 30−35 mN/m in the Langmuir experiment. Therefore, the pressure of 30 mN/m seems to be appropriate for a deposition in the planned biosensor construction. The monolayer stability is known to be a very

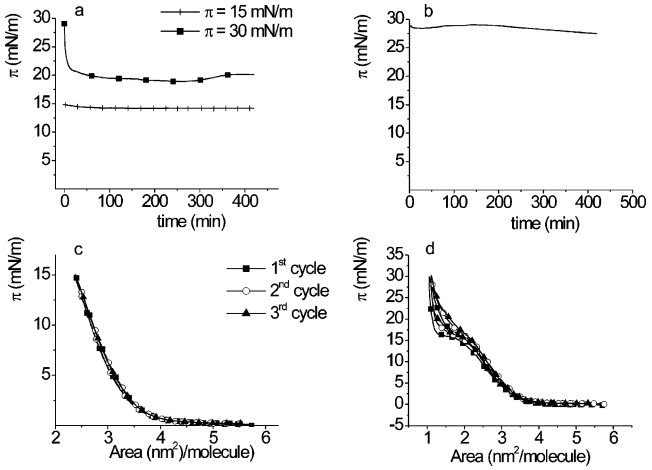

Figure 4. Stability measurements: (a) $\pi-t$ dependencies for pure gramicidin monolayer, (b) $\pi-t$ dependence for the mixed monolayer gramicidin/F6H18 at X(gramicidin) = 0.1, and (c,d) compression/expansion cycles for pure gramicidin monolayer.

important factor for the Langmuir−Blodgett transfer, and therefore we have focused our attention on examining the stability of mixed monolayers of X(gramicidin) = 0.1 in addition to films from pure components. For this purpose, two kinds of experiments have been performed.

In static stability experiments, the investigated monolayers were compressed to a particular surface pressure value (15 and 30 mN/m). Afterward, the compression was stopped and free decay of π value versus time was monitored (Figure 4a and b). In a dynamic stability experiment, the subsequent compression−expansion cycles (monolayers were compressed to the π values mentioned above) were carried out (Figure 4c and d).

Both kinds of experiments evidence that the gramicidin monolayer is very stable in the region preceding the transition. At higher pressures, an initial drop of surface pressure is observed; however, within the first 20 min, it levels off at 19 mN/m, and no further decrease in π can be noticed for a long period of time. This proves that gramicidin can be transferred onto solid supports from a high-pressure region; however, the observed fall in π (from 30 to 19 mN/m) indicates that gramicidin will become inactive with regards to ion conduction.

High stability of gramicidin monolayers at 15 mN/m has also been proved by dynamic stability measurements. As it can be seen, for a monolayer compressed to 15 mN/m, three compression/expansion cycles overlap (Figure 4c). However, when the monolayer is compressed to 30 mN/m (Figure 4d), the hysteresis loops are visible, but only in the transition region. Dynamic stability experiments performed at both investigated surface pressures evidence that gramicidin is stable in monolayers at the air/water interface and does not undergo dissolution into the bulk phase.

Our results are in accordance with those published by Van Mau et al.,[24] who reported a stable monolayer formation from gramicidin spread onto the air/water interface from a chloroform−methanol solution. Because of the peptide structure (gramicidin A is built of hydrophobic amino acids), gramicidin A molecules do not undergo dissolution from a monolayer into bulk water. Although the solubility of gramicidin in water is negligible, it has been reported that it is possible to prepare its very diluted aqueous solution (10^{-7} M).[28] However, the presence of gramicidin in the bulk phase does not lead to the appearance of a noticeable amount of gramicidin at the interface.[28]

Regarding the stability experiments for the mixed monolayer, a different tendency has been observed (Figure 4b). At the beginning of the experiment, a minute fall of the π value from 30 to ca. 29 mN/m takes place, but later on, during many hours of observation, the surface pressure remains constant within the limits of error. As it has been indicated above, the surface pressure of ca. 30 mN/m corresponds to the conditions existing in cellular membranes, and at such a pressure gramicidin is in its vertical, bio-active conformation. This stabilizing effect of SFA molecules on gramicidin is a crucial factor in the planned deposition of these materials and the design of gramicidin A-based stochastic biosensors.

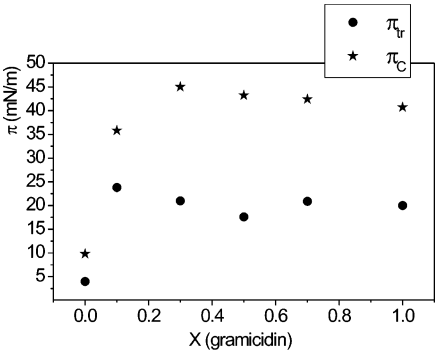

Figure 5. Dependencies of collapse pressure (π_c) and transition pressure (π_{tr}) versus the mole fraction of gramicidin for the system gramicidin/F6H18.

Discussion

According to the 2D-phase rule,[49−51] if two components of the mixed Langmuir monolayer are miscible, only one collapse pressure is observed, the value of which lies between values characteristic of pure monolayers. The same rule can be applied to phase transitions. From the $C_S^{-1}-\pi$ plots, the exact values of the collapse and transition pressures (π_c and π_{tr}, respectively) were obtained. The plots of collapse and transition pressures versus the mole fraction of gramicidin are drawn in Figure 5.

In the case of both π_c and π_{tr}, it occurs that the values observed for mixtures are greater than those characteristic of pure monolayers. This implies that the interactions between unlike molecules (gramicidin/SFA) are more attractive as compared to the interactions between like molecules (SFA−SFA and gramicidin−gramicidin). The highest transition pressure value (ca. 25 mN/m) is typical for the mixtures containing 0.1 mole fraction of gramicidin, whereas the highest collapse pressure (ca. 45 mN/m) is found for a gramicidin mole fraction of 0.3 mN/m. For the greater peptide proportion, the values of π_c and π_{tr} approach those for the pure gramicidin monolayer.

Another criterion, which can shed light on the mutual miscibility of gramicidin A and the investigated SFA, is the average mean molecular area, A_{12}, which is defined as follows:[50]

$$A_{12} = A_1X_1 + A_2X_2 \qquad (1)$$

where A_1 (A_2) stands for the molecular area of the single component monolayer at the same surface pressure as it is applied to determine A_{12} in the mixture, while X_1 and X_2 are the mole fractions of components 1 and 2 in the mixed film. The linear dependence of A_{12} versus mole fraction of one component can indicate either ideal miscibility or complete immiscibility.[49,50] Positive deviations indicate that the interactions between unlike molecules in a mixed monolayer are less attractive than those between like molecules in a pure monolayer, whereas negative deviations prove the reverse tendency. The plots of A_{12} versus X(gramicidin) are shown in Figure 6.

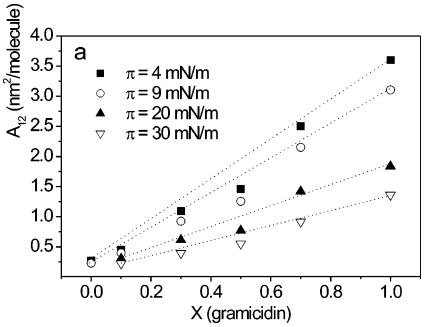

Figure 6. Average mean molecular area (A_{12}) versus gramicidin mole fraction.

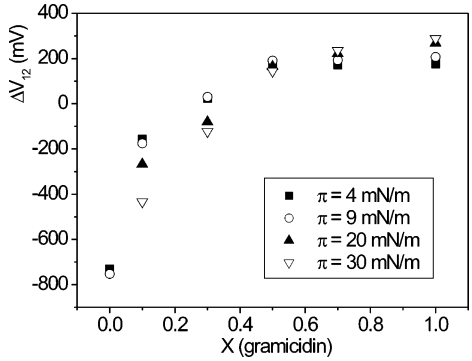

Figure 7. Average electric surface potential ΔV_{12} versus gramicidin mole fraction.

The values of A_{12} were taken at the following surface pressure values: 4, 9, 20, and 30 mN/m. The first value corresponds to the phase transition region in a $\pi-A$ isotherm of pure SFA monolayer, whereas the second value corresponds to the vicinity of its collapse pressure. The value of 20 mN/m is characteristic of the transition region for the gramicidin monolayer, whereas at 30 mN/m gramicidin is in its vertical orientation. As is evident from Figure 6, for the investigated system negative deviations from linearity can be observed. They are stronger at lower surface pressure values and diminish upon increasing π. Negative deviations corroborate that the interactions between gramicidin and SFA molecules are more attractive than the interactions between like molecules in pure one-component films. From the plots of $A_{12}-X$(gramicidin), it can be inferred that gramicidin mixes with the investigated SFA in the whole range of composition.

Similarly to $\pi-A$ isotherms, also $\Delta V-A$ dependencies for mixtures can be interpreted according to the additivity rule,[53,54] and in analogy to A_{12}, ΔV_{12} can be defined as follows:

$$\Delta V_{12} = \Delta V_1 X_1 + \Delta V_2 X_2 \qquad (2)$$

where ΔV_{12} is the average electric surface potential change per molecule in the mixed monolayer, $\Delta V_1(\Delta V_2)$ stands for the molecular area of the single component monolayer at the same surface pressure as is applied to determine ΔV_{12} in the mixture, while X_1 and X_2 are mole fractions of components 1 and 2, respectively.

For an ideal mixture of noninteracting molecules, the plot of $\Delta V-X_1(X_2)$ is linear, whereas for electrically interacting molecules, deviations from linearity are observed. The dependencies of ΔV_{12} versus X(gramicidin) are presented in Figure 7. Similarly to $A_{12}-X$(gramicidin) plots, the $\Delta V_{12}-X$(gramicidin) curves were recorded at the following π values: 4, 9, 20, and 30 mN/m. However, regardless of the value of π, significant positive deviations from linearity are visible in the course of $\Delta V_{12}-X$(gramicidin) plots. This indicates that there exists a strong dipolar interaction between gramicidin and SFA molecules in mixed Langmuir monolayers, and the shape of the $\Delta V_{12}-X$(gramicidin) curves again proves mutual miscibility between both components.

Our results of gramicidin−SFA mixtures are exceptional with respect to the existence of a very strong interaction between monolayer dipoles. To the best of our knowledge, such an effect has never been observed so far in the investigated gramicidin-containing mixtures. The observed strong interactions result from the opposite direction of dipole moment vectors of both components at the air/water interface, which is evidenced by the opposite sign of the measured ΔV (negative for SFA and positive for gramicidin). The ordered, antiparallel orientation of the dipole moments in the mixed monolayers gramicidin/SFA is responsible for the observed high stability of the system. SFA molecules are thus perfect candidates for gramicidin incorporation for the biosensor construction.

Conclusions

Gramicidin A forms mixed monolayers with the investigated semifluorinated tetracosane within the whole range of molar fractions. The analysis of the collapse pressure values indicates that mixed films are more stable as compared to the monolayers of pure components. Even a small addition of gramicidin to the SFA monolayer (X(gramicidin) = 0.1) affects profoundly the monolayer stability, and a significant increase of the collapse pressure value from ca. 10 to 35 mN/m has been observed. The mixture of X(gramicidin) = 0.1 has been investigated thoroughly due to its potential application for the biosensor design. For pure gramicidin A monolayer compressed to a high surface pressure value (30 mN/m, which corresponds to the pressure in natural membranes, where gramicidin is in its ion-permeable, vertical conformation), a considerable fall in surface pressure (to ca. 19 mN/m, where the peptide is in its nonactive horizontal conformation) has been noticed in the first minutes. However, when gramicidin is scattered in a monolayer from SFA, film stability increases significantly; that is, the observed decrease in surface pressure was only 1 mN/m (from 30 to 29 mN/m). This experiment proves that in the mixture with SFA, gramicidin can be transferred onto solid supports without losing its ion-conducting properties. Significant deviations from linearity, observed in the plots of $A_{12}-X$(gramicidin) and $\Delta V_{12}-X$(gramicidin), together with high stability of mixed monolayers were explained by the existence of strong dipole−dipole interactions, due to dipole moment vectors of SFA and gramicidin aligned aniparallel at the interface. SFA molecules have been proven to be good candidates for incorporating gramicidin for a biosensor construction.

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