

Investigations of the Orientation of a Membrane Peptide by Sum Frequency Spectroscopy

Gibum Kim, Marc C. Gurau, Soon-Mi Lim, and Paul S. Cremer*

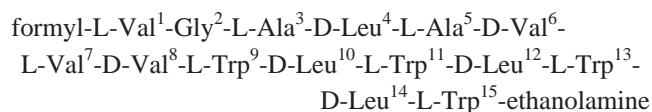
Department of Chemistry, Texas A&M University, P.O. Box 30012, College Station, Texas 77843-3012

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Sum frequency spectra of gramicidin A at the air/water interface in partially deuterated dimyristoylphosphatidylcholine monolayers were collected as a function of surface pressure and mole fraction of the peptide. Reorganization of the lipid and peptide molecules were followed between 5 and 35 mN/m. Selective deuteration of the lipid enabled independent observation of the ordering of the lipid tail and headgroup regions. The results from pure lipid monolayer studies revealed that both portions of the amphiphile rearranged roughly in tandem as a function of pressure. When the membrane peptide was added to the monolayer, changes in its orientation were noted at mole fractions below 0.5, but not above this value. Specifically, two sets of data for the peptide could be obtained. One was for the aliphatic residues and the other for the Trp residues as each gave a distinct sum frequency signal in the CH stretch range. The aliphatic residues, which are dispersed throughout gramicidin, revealed that the whole peptide could readily track changes in lipid orientation at 0.1 mole fraction but did so less readily at 0.4 mole fraction. On the other hand, the Trp residues, which all reside near the C-terminus and interact strongly with the lipid headgroups, showed a greater propensity to track lipid reorientation at the higher mole fraction. These results indicated that changes in the C-terminus probably precede realignment in other parts of the membrane peptide at higher peptide concentration. Furthermore, peptide–peptide interactions probably inhibit gramicidin A reorientation.

Introduction

Gramicidin, secreted from *Bacillus brevis*, is considered a model peptide for studies of interfacial biomaterials due to the simplicity of its structure.^{1,2} Four different types of gramicidin are known to exist with different sequences and structures. Gramicidin S is cyclic whereas A, B, and C are linear pentadecapeptides. The difference among A, B, and C is in the amino acid residue at position 11. Gramicidin A has a tryptophan, B has a phenylalanine, and C has a tyrosine at this position.³ The sequence of gramicidin A was first reported in 1965 and is shown below:⁴



The peptide is composed of hydrophobic residues and is capped at its ends, which prevents it from becoming charged as the pH is altered. Four Trp residues lie near the C terminus and come in direct contact with the headgroup region of the membrane. The orientation of these residues is believed to play a crucial role in channel structure and cation transfer.^{5–8} 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.⁹ Increasing the dipole moment of Trp by selective fluorination of the six membered ring has been shown to increase ion-channel conductivity.¹⁰ The existence of two different hydrogen bonding motifs for Trp residues has been demonstrated in previous studies.^{8,11,12} In one case, the N–H on Trp was shown

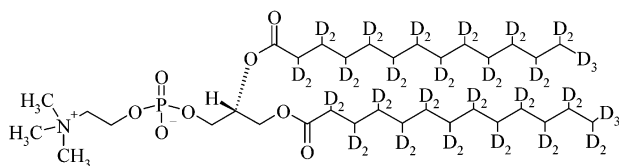
to hydrogen bond with surrounding water molecules and in the other with the carbonyl moieties on the phospholipids.

Alternating L and D residues in the sequence allow for the formation of at least two different dimer conformations, a double helix and a helical dimer.¹³ The helical dimer, which is believed by many investigators to function as an ion channel in lipid bilayers,¹⁴ 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 is the form that has most often been observed in X-ray crystal structure studies.² It has been postulated that this is the dimer structure that forms in Langmuir monolayers of pure gramicidin A at the air/water interface upon compression.¹³

The impetus to study gramicidin monolayers has not only been biological, but also for the development of biomaterials and sensors. In this case pure gramicidin films or films with high ratios of gramicidin to lipid are often employed. Orientational changes in gramicidin A in pure peptide monolayers spread at the air/water interface have been previously studied by various techniques including polarization modulation FTIR (PM-FTIR),¹⁵ ellipsometry,¹⁶ X-ray reflectivity,¹⁶ and surface tension measurements.^{17,18} The data indicated that the tilt angle of the helical axis was not significantly dependent on the surface pressure as determined by PM-FTIR.¹⁵ However, when gramicidin A was mixed with partially deuterated dimyristoylphosphatidylcholine (DMPC-*d*₅₄, see Chart 1) at a mole fraction of 0.18 for the peptide, the tilt angle of the helix changed from 90° to 25° with respect to the surface normal as the surface pressure was increased from 5.7 to 40 mN/m.¹⁵

Pressure–area (π –*A*) diagram studies of gramicidin A/phospholipid mixtures have been performed at a variety of different molar ratios of gramicidin to lipid.¹⁷ Isotherms of gramicidin A monolayers in the absence of lipid showed a characteristic

* To whom correspondence should be addressed. Tel: 979-862-1200. Fax: 979-845-7561. E-mail: cremer@mail.chem.tamu.edu.

CHART 1. Molecular Structure of DMPC-*d*₅₄

deflection point at about 12–15 mN/m before reaching a stiff incompressible state as the surface pressure was increased. This deflection point disappeared when the Trp residue at position 9 was replaced with Phe.¹⁷ Although the exact cause of the deflection in the π -A diagram is still not entirely clear, one possibility may be the irreversible dimerization of gramicidin. Indeed, X-ray reflectivity measurements have shown that the thickness of the compact gramicidin film on water is roughly 27 Å.¹⁶ This value corresponds well with the known dimensions of the double helix from X-ray crystal structure studies (length 26–31 Å, width 15–16 Å), if the dimer is tilted by at least 60° with respect to the surface normal.^{2,15} Significantly, it was shown by surface tension measurements that the deflection point in the π -A diagrams disappeared as the mole fraction of gramicidin A in the phospholipid monolayer was decreased below 0.5.¹⁷ In this case the π -A diagrams displayed reversible behavior and did not appear to depend strongly on the specific type of phospholipid molecule used (e.g., Egg PC or DOPE).

In the studies presented here, we have employed vibrational sum frequency spectroscopy (VSFS) to simultaneously investigate orientational changes in both lipid and gramicidin A molecules at the air/water interface as a function of surface pressure and peptide/lipid mole ratio. The purpose was to gain a better understanding of this molecule's behavior in biomaterials applications where high mole ratios of peptide would be employed. Our strategy was to study the Trp rich C-terminus independently from the remainder of the molecule. This was possible because the aromatic CH stretch frequency from Trp is well separated from the aliphatic CH stretch signal from the other hydrophobic residues. The gramicidin was incorporated into DMPC-*d*₅₄ monolayers. Deuteration of the lipid tails allowed for direct observation of the choline methyl stretches from the lipid headgroups, which were in closest contact with the Trp residues.

Unlike traditional vibrational spectroscopies, VSFS is a surface specific technique that is very sensitive to orientational rearrangements.^{19–21} Our results showed that gramicidin A rearranged some degree of order even in the absence of lipids. In this case the peptide showed no significant changes in orientation as a function of pressure. However, upon compression of monolayers with peptide mole fractions below 0.5, dramatic changes in orientation were observed. These changes revealed that the Trp rich C-terminus more closely tracked the orientation of the lipid molecules at 0.4 mole fraction of the peptide compared with the rest of the gramicidin. Lowering the mole fraction of gramicidin to 0.1 also seemed to allow the rest of the molecule to more readily rearrange with the lipids. Furthermore, the head and tail moieties of the lipids were shown to reorient approximately in concert with each other as a function of pressure in pure lipid monolayers.

Experimental Section

VSFS. The 1064 nm radiation source used in all VSFS experiments came from a mode-locked Nd:YAG laser (PY61c, Continuum, Santa Clara, CA) operated at a 20 Hz repetition rate with a peak width of 21 ps. This beam was used to pump

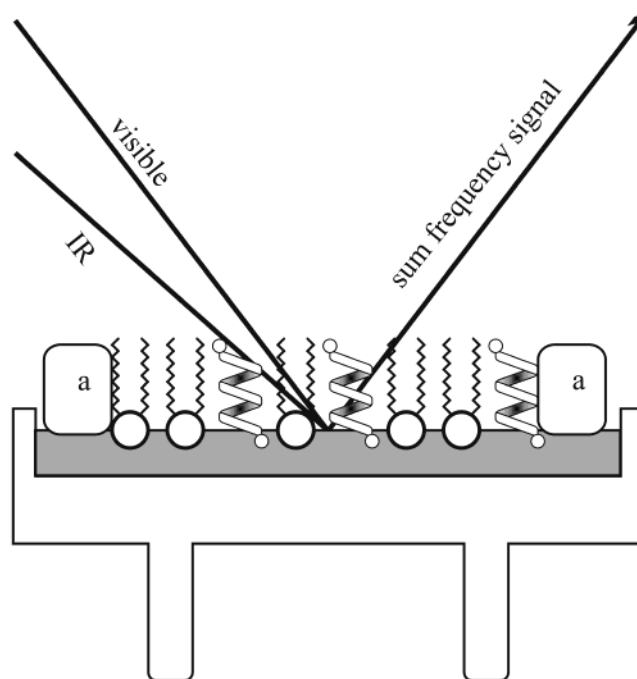


Figure 1. Experimental setup for the gramicidin monolayer study. Modulation of the lateral surface pressure was achieved by moving Teflon barriers across the surface of a Langmuir trough. The letter “a” represents the movable barriers. This scheme is not drawn to scale.

an optical parametric generation/amplification (OPG/OPA) stage (Laser Vision, Bellevue, WA) to produce a tunable IR beam from 2800 to 3100 cm⁻¹ in addition to a frequency doubled beam at 532 nm. Figure 1 is a schematic diagram of the experimental setup. The IR and the visible beams were overlapped at the air/water interface with incident angles of 51° and 42°, respectively, with respect to the surface normal.

The theoretical details of VSFS have been described elsewhere.^{19–21} Sum frequency signal intensity, I_{SF} , is proportional to the square of the second-order nonlinear susceptibility, $\chi^{(2)}$. This susceptibility tensor can be described by a frequency dependent resonant term, $\chi_{\text{R}}^{(2)}$, and a frequency independent nonresonant term, $\chi_{\text{NR}}^{(2)}$. The intensity of the sum frequency signal can be written as

$$I_{\text{SF}} \propto |\chi^{(2)}|^2 I_{\text{IR}} I_{\text{VIS}} = |\chi_{\text{R}}^{(2)} + \chi_{\text{NR}}^{(2)}|^2 I_{\text{IR}} I_{\text{VIS}} \quad (1)$$

$$\chi_{\text{R}}^{(2)} = \sum_n \frac{A_n}{\omega_{\text{IR}} - \omega_n + i\Gamma_n} \quad (2)$$

where I_{VIS} and I_{IR} are the intensities of the input visible and infrared beams. A_n is the oscillator strength of the n th resonant mode and includes the density of oscillators, their orientational vector average, as well as the strength of the IR and Raman transition moments. ω_{IR} , ω_n , and Γ_n represent the frequency of the incoming IR beam, the frequency of the n th resonant mode as well as its peak width. All spectra in this study were taken with the ssp polarization combination, which refers to the sum frequency, visible, and infrared beams, respectively. Spectra with a complementary polarization combination, sps, were attempted, but the signal was quite weak and the results are, therefore, not included here. The weak signal for sps data with corresponding stronger signal for ssp was a strong indication that the majority of the long-range molecular alignment was normal to the interface. Each data set was normalized to spectra taken from a piece of Y-cut crystalline quartz. Data were fit with a Voigt

profile to yield the oscillator strength of individual resonances. This took into account both homogeneous and inhomogeneous line broadening.

Materials. The water used in preparing buffers for these experiments was obtained from a NANOpure Ultrapure Water System (Barnstead, Dubuque, IA) and had a minimum resistivity of $18 \text{ M}\Omega\cdot\text{cm}$. PBS buffers at pH 7.0 were made with 100 mM Na_2HPO_4 and 40 mM NaH_2PO_4 in the presence of 100 mM NaCl. The buffer was diluted by a factor of 4 to obtain a solution with a final ionic strength of 110 mM. Gramicidin A (90 mol % purity with the remainder consisting of gramicidin B and C) was purchased from Fluka (Milwaukee, WI) and used as received. A Langmuir trough (Model 601 M, Nima Technology Ltd., Coventry, England) equipped with a Wilhelmy plate was used to measure the surface pressure after spreading the monolayers. The surface pressure was adjusted by changing the area of the air/water interface with two movable Teflon barriers. DMPC- d_{54} (Chart 1), DMPC, and NBD-DPPE (1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)) were purchased from Avanti Polar Lipid (Alabaster, AL) and used as received. The gramicidin A and phospholipid monolayers were prepared on PBS buffer solutions in the Langmuir trough by spreading droplets from 1 mg/mL chloroform solutions containing the appropriate dissolved components. The surface pressure for each system was calibrated by setting the value of the pure buffer surface to 0 mN/m before spreading the monolayer. Spectra were taken in increments of 5 mN/m as the surface pressure was raised sequentially from 5 to 35 mN/m. In the figures presented here, oscillator strength data are plotted using all seven measurements, but full spectra are only shown in increments of 10 mN/m to avoid crowding in the diagrams.

Results

Representative π - A diagrams of gramicidin A in DMPC- d_{54} monolayers with mole fractions ranging from 0.0 to 1.0 are shown in Figure 2a. Figure 2b plots the first derivative of polynomial fits to these data for easier identification of pertinent features. A pure DMPC- d_{54} monolayer showed smooth behavior with a slow decrease in the slope of the curve upon monolayer compression (curve 0.0 in Figure 2b). When the mole fraction of the peptide was 0.4 or less (curves 0.1 and 0.4 in Figure 2a), smooth π - A behavior was also observed. In this case a slow increase in the slope was seen for the first derivative curve as the monolayers were compressed (Figure 2b). A deflection point was finally visible, although not well pronounced, at a mole fraction of 0.5 (curve 0.5 in Figure 2a) in agreement with literature measurements.^{17,22} In the case of a pure gramicidin monolayer (curve 1.0 in Figure 2a), the characteristic deflection can be seen quite well around 12–16 mN/m. This led to a clear maximum in the first derivative data (curve 1.0 in Figure 2b). The π - A diagrams were irreversible for peptide mole fractions of 0.5 and above, but reversible below this value. Epifluorescence experiments with DMPC- d_{54} /gramicidin monolayers containing 1 mol % NBD-labeled lipids were carried out for each molar ratio. These showed that under all conditions the monolayers were homogeneous down to the diffraction limit, as judged by fluorescence microscopy. Such results agreed with previous observations.^{23,24}

VSFS spectra of a pure DMPC- d_{54} monolayer as a function of surface pressure between 5 and 35 mN/m are shown in Figure 3a. The data show a single feature at 2967 cm^{-1} (Figure 3a). This peak originated from the undeuterated headgroup and the frequency matched best with the choline moiety as opposed to

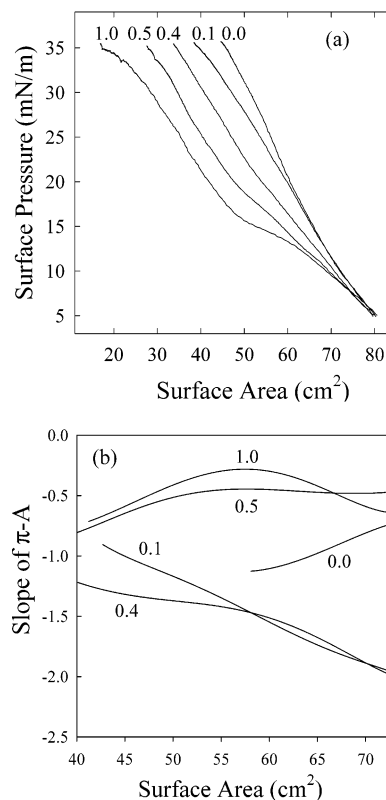


Figure 2. (a) π -area diagrams and (b) first derivatives of fits to these for DMPC- d_{54} /gramicidin A mixed monolayers on a Langmuir trough as the lateral pressure was varied.

the CH or CH_2 modes in Raman data (Chart 1).²⁵ To ascertain that sum frequency signal in this region of the spectrum may emanate from methyl groups bound to quaternary nitrogens, control experiments were performed with selectively deuterated dimethyldidodecylammonium bromide monolayers where only the two methyl groups attached to the nitrogen remained undeuterated. These spectra confirmed the generation of VSFS signal at this frequency.

Fitted oscillator strength values for the DMPC- d_{54} data are shown in Figure 3b. This allowed quantification of the increase in oscillator strength as the monolayer was compressed. Curiously, the data seem to indicate that the rise in oscillator strength was roughly linear with the increase in surface pressure. Data were also taken for hydrogenated DMPC. In this case the well-known CH_3 symmetric stretch feature at 2882 cm^{-1} from the terminal methyl groups of the alkyl chains dominated the spectra (data not shown).^{19,26} Fitted data from this peak as a function of pressure are shown in Figure 3c. The data revealed the increase in the oscillator strength of the tail feature was also approximately linear with pressure. Monolayer compression effects sum frequency spectra due to changes in the alignment of dipoles as well as through increases in their number density.^{19,26} Although both may occur, it is well established that the tail groups become better organized upon compression.^{19,26} The data in the present study clearly indicate that lipid headgroups also undergo reorientation and align with the surface normal approximately in concert with the tails as a function of pressure.

Adding a small concentration of gramicidin A to the DMPC- d_{54} monolayer (0.1 mole fraction) caused a second peak to become visible in the data at 2884 cm^{-1} (Figure 4a). This feature, which was most likely the symmetric stretch of methyl groups, was due to the alignment of aliphatic moieties in the gramicidin A side chains. A similar aliphatic CH stretch feature

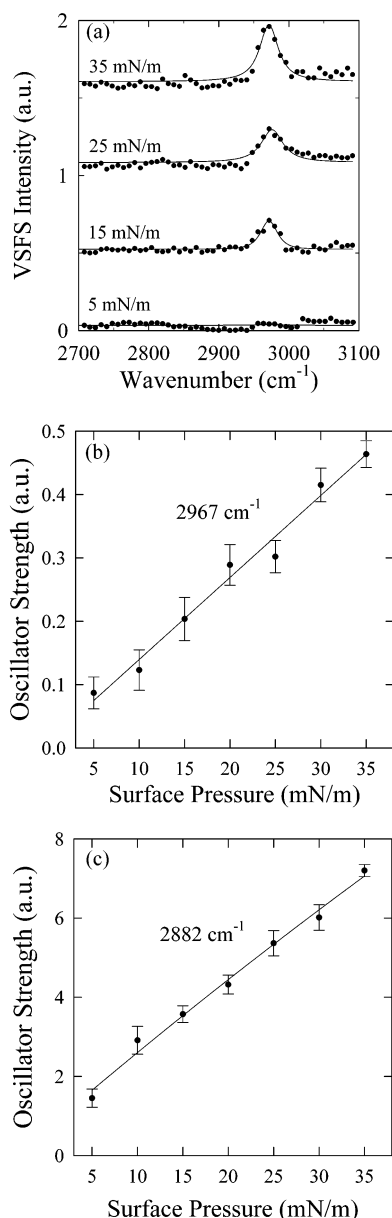


Figure 3. (a) Sum frequency spectra from a DMPC- d_{54} monolayer at various surface pressures. All spectra were taken at pH 7.0 over a phosphate buffer solution going from low surface pressure to high. The solid lines represent least-squares fits to the data using a Voigt profile. (b) Plot of the values of the oscillator strengths (A_n) for the 2967 cm^{-1} peak from (a) at various lateral surface pressures. The lines drawn through the data are least-squares fits with a second-order polynomial. (c) Fitted values of oscillator strength (A_n) for the 2882 cm^{-1} peak from a hydrogenated DMPC monolayer as a function of lateral surface pressure. The solid line represents a least-squares fit to the data with a second-order polynomial.

in VSFS has already been found in interfacial protein studies with lysozyme.²⁷ Furthermore, control studies with polyalanine and polyglycine monolayers gave rise to this peak in addition to a feature at 2943 cm^{-1} , which was too weak to observe under these low concentration conditions.²⁷ The 2884 cm^{-1} peak from gramicidin and the 2967 cm^{-1} feature from the partially deuterated lipids showed opposite behavior upon compression. Namely, the signal from the 2884 cm^{-1} feature attenuated as the surface pressure was increased, while the 2967 cm^{-1} feature rose. The oscillator strengths of both features were fit by a Voigt function and the fitted oscillator strengths are plotted in Figure 4b. These results confirmed that the lipid headgroup signal

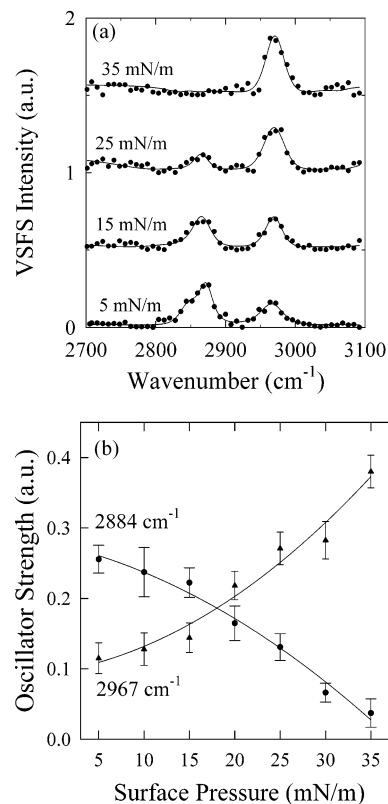


Figure 4. (a) Sum frequency spectra from a DMPC- d_{54} /gramicidin A monolayer at various surface pressures. The mole fraction of gramicidin was 0.1. All spectra were taken at pH 7.0 over a phosphate buffer solution going from low surface pressure to high. The solid lines represent least-squares fits to the data using a Voigt profile. (b) Plots of the values of the oscillator strengths (A_n) for the 2884 and 2967 cm^{-1} peaks from (a) versus lateral surface pressure. The lines drawn through the data are least-squares fits with a second-order polynomial.

increased as a function of pressure concomitantly with the decrease in intensity from the aliphatic residues. Since the strongest VSFS intensity from the peaks at 2884 cm^{-1} was observed at low pressure, it could be inferred that the direction of the net dipole moment from the aliphatic residues was most likely perpendicular to the helical axis. This is consistent with X-ray crystal structure data for the peptide in a membrane.^{2,28} Furthermore, at low surface pressure, the tilt angle of the helix of gramicidin A is known to be close to 90° with respect to the surface normal from work at 0.18 mole fraction of gramicidin in the lipid monolayer.¹⁵ The helical axis tilts upward upon monolayer compression, which caused the peak to attenuate as the aliphatic residues reoriented away from the surface normal.

The results in Figure 5a with 0.4 mole fraction gramicidin A showed evidence for several more gramicidin features. The peaks at 2884, 2943, and 3064 cm^{-1} as well as a low frequency shoulder on the 2884 cm^{-1} peak, matched frequencies previously observed in VSFS studies for other polypeptides.²⁷ The appearance of the additional peaks as well as the increase in the strength of the 2884 cm^{-1} can be attributed to the increase in number density of gramicidin molecules in the monolayer compared with the 0.1 mole fraction results. On the other hand, the 2967 cm^{-1} was still present, but because the mole fraction of lipid was decreased, it appeared somewhat weaker. As indicated above, the features at 2943 and 2884 cm^{-1} were due to the alignment of aliphatic moieties in the gramicidin A side chains.²⁷ The feature at 3064 cm^{-1} can be assigned to an aromatic CH stretch from the Trp residues. This feature has also been seen in lysozyme studies, although Trp is not the only

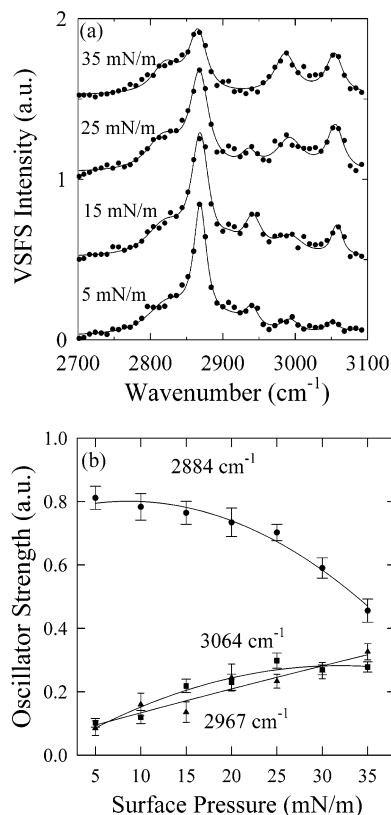


Figure 5. (a) Sum frequency spectra from a DMPC- d_{54} /gramicidin A monolayer at various surface pressures. The mole fraction of gramicidin was 0.4. All spectra were taken at pH 7.0 over a phosphate buffer solution going from low surface pressure to high. The solid lines represent least-squares fits to the data using a Voigt profile. (b) Plots of the values of the oscillator strengths (A_n) for the 2884, 2967, and 3064 cm^{-1} peaks from (a) versus lateral surface pressure. The lines drawn through the data are least-squares fits with a second-order polynomial.

aromatic residue in this protein. As verification of the 3064 cm^{-1} assignment in gramicidin A, control spectra with L-tryptophan at the air/solution interface were taken.²⁹ It was found that this system also gave rise to intensity near 3064 cm^{-1} (data not shown). On the other hand, polyalanine and polyglycine monolayers on water do not give rise to this peak.²⁷

The strongest VSFS intensities from the peaks at 2884 and 2943 cm^{-1} were observed under low-pressure conditions. As the monolayer was compressed, the helical axis tilted upward and the aliphatic peak intensities decreased.¹⁵ On the other hand, the aromatic CH stretch at 3064 cm^{-1} increased as the monolayer was compressed. This indicated that the transition dipole from the Trp residues was not perpendicular to the helical axis, but probably closer to parallel to it in agreement with Raman,^{7,9} X-ray crystal structure,²⁸ and NMR data.³⁰ The trends in both the 2884 and 3064 cm^{-1} features were verified on the basis of the values of their fitted oscillator strengths (Figure 5b).

Sum frequency spectra of gramicidin A at 0.5 mole fraction in the DMPC- d_{54} monolayer are shown in Figure 6a. An overall increase in intensity of the gramicidin features with respect to the data in Figure 5a again reflected an increase in the number density of this species at the interface (note change in y-axis scale). It appeared that significant changes in peptide orientation did not take place as a function of surface pressure under these conditions, based on the values of fitted oscillator strengths of the CH peaks (Figure 6b). Changes might still have occurred

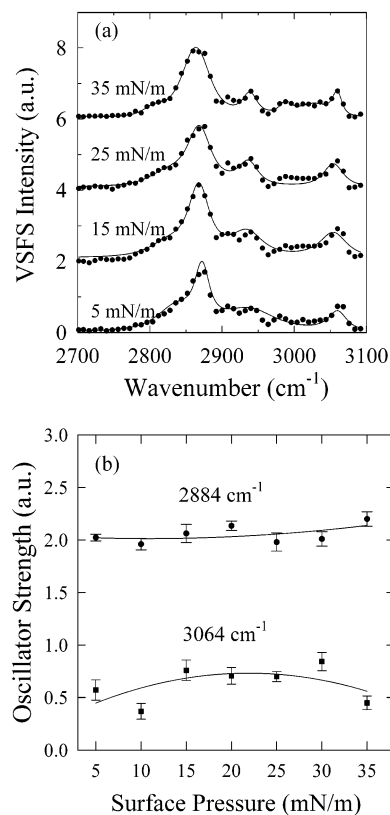


Figure 6. (a) Sum frequency spectra from a monolayer of an equal molar mixture of DMPC- d_{54} /gramicidin A at various surface pressures. All spectra were taken at pH 7.0 over a phosphate buffer solution going from low surface pressure to high. The solid lines represent least-squares fits to the data using a Voigt profile. (b) Plots of the values of the oscillator strengths (A_n) for the 2884 and 3064 cm^{-1} peaks from (a) versus lateral surface pressure. The lines drawn through the data are least-squares fits with a second-order polynomial.

in the 2967 cm^{-1} feature; however, this peak was quite weak and not suitable to fit quantitatively.

Figure 7a shows VSFS spectra taken from a pure gramicidin A monolayer at surface pressures between 5 and 35 mN/m. The fitted values of the oscillator strength for the 2884 and 3064 cm^{-1} peaks are given in Figure 7b. These again showed only minimal evidence for changes in the CH stretch intensities as the monolayer was compressed. The lack of reorientation correlated well with previous studies of pure gramicidin A monolayers by PM-FTIR and X-ray reflectivity.^{15,16}

Discussion

From Figure 2a,b it can be seen that when the mole fraction of gramicidin A in the monolayers was 0.5, a deflection point appeared in the π - A diagrams. The mole fraction where significant orientational changes ceased to occur in the VSFS spectra coincided with this (Figures 6 and 7). For pure gramicidin monolayers, previous X-ray reflectivity studies have shown that double-stranded helices were probably formed at high pressure after proceeding through the phase transition.¹⁶ The irreversible process of dimer formation would appear to prevent significant changes in the orientation of gramicidin A molecules. On the other hand, when sufficient lipid concentrations were introduced (peptide mole fraction < 0.5), the lipid molecules significantly hindered dimer formation. As a result, the peptides tilted upward in conjunction with the lipid molecules as the entire system was compressed.

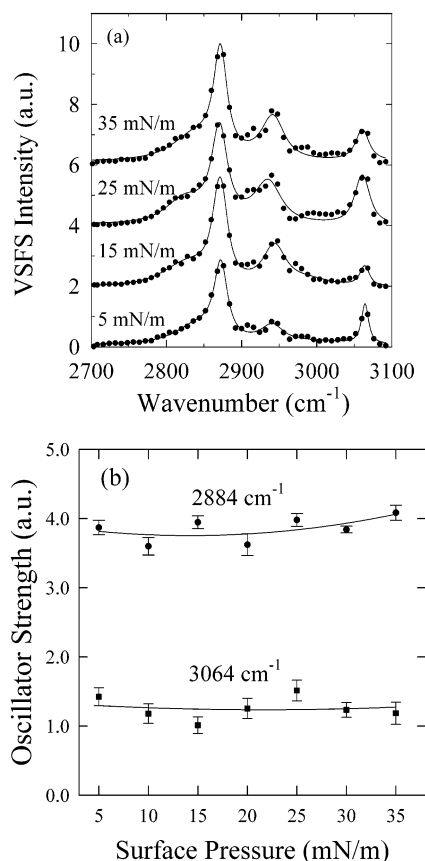


Figure 7. (a) Sum frequency spectra from a pure gramicidin A monolayer at various surface pressures. All spectra were taken at pH 7.0 over a phosphate buffer solution going from low surface pressure to high. The solid lines represent least-squares fits to the data using a Voigt profile. (b) Plots of the values of the oscillator strengths (A_n) for the 2884 and 3064 cm^{-1} peaks in (a) versus lateral surface pressure. The lines drawn through the data are least-squares fits with a second-order polynomial.

Compression of the monolayer with 0.4 mole fraction of gramicidin A led to a decrease in VSFS signal from the aliphatic peptide moieties at 2884 cm^{-1} , while the opposite trend was observed for the 3064 cm^{-1} Trp feature as well as the 2967 cm^{-1} peak from the lipid headgroups (Figure 5). Under these conditions, neither the peak from the aliphatic residues nor the one from the Trp residues completely tracked the changes seen in the lipid as a function of pressure. Indeed, the lipid headgroup signal appeared to vary approximately linearly with lateral surface pressure. By contrast, the aromatic feature from the Trp residues displayed more reorientation at lower surface pressures, while the aliphatic CH intensity from the entire gramicidin indicated that reorientation only occurred at higher surface pressures. These results may be due to different interactions between the lipid heads and the Trp rich C-terminus on the one hand and the lipid tails and the aliphatic rich N-terminus on the other. Indeed, Trp residues in gramicidin have been shown to stabilize gramicidin structure in membranes.^{5,31} The indole moiety may, therefore, have stronger interactions with lipid headgroups and cause the C-terminus to reorient more easily, while the rest of the molecule follows only at increased pressure. Such results are almost reminiscent of work by Eienthal and co-workers where alkyl chains in a fatty acid monolayer were seen to reorient more facily than their $\text{C}\equiv\text{N}$ headgroups as a function of pressure.³² In the present case, however, it is the peptide's interactions with the lipid headgroups that govern the movement.

At 0.1 mole fraction of gramicidin (Figure 4), the 2884 cm^{-1} feature showed somewhat different behavior. In contrast with higher peptide concentrations, the peak from the aliphatic residues began to attenuate at lower pressure and disappeared completely by 35 mN/m (Figure 4). Such behavior seemed to indicate that when the peptide was sufficiently solvated by surrounding lipid molecules, changes in aliphatic residues tracked more closely to those of the lipids. Since this was not the case at 0.4 mole fraction, it seems reasonable to postulate that substantial peptide-peptide interactions must still have existed in the latter case. Indeed, the significant residual signal at 2884 cm^{-1} with 0.4 mole fraction gramicidin at 35 mN/m surface pressure (Figure 5b) may even be an indication that not all of the peptide molecules underwent complete reorientation under these conditions.

Conclusions

Reorientation of gramicidin A and DMPC- d_{54} mixed monolayers at the air/water interface was monitored as a function of two-dimensional pressure. It was found in pure DMPC monolayers that the ordering of lipid headgroups tracked those of the alkyl chains. In the presence of lower concentrations of peptide, the whole gramicidin molecule appeared to reorient with the lipids. At higher concentrations, however, the Trp rich C terminus seemed to reorient with the lipid before the rest of the peptide. Finally, when the mole fraction of peptide was raised to 0.5 or above, no significant change in peptide orientation could be observed.

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