Lipid-alamethicin interactions influence alamethicin orientation

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ABSTRACT Whereas the barrel-stave configuration is accepted by most investigators as a good description of the conducting state of alamethicin, there are conflicting interpretations on its nonconducting state; in the absence of an applied field, some found alamethicin molecules on the membrane surface, but others found them incorporated in the hydrophobic core of the membrane. This problem is resolved by the discovery of a phase-transitionlike behavior of alamethicin in the membrane. As a function of lipid/peptide ratio L/P and the chemical potential of water μ, alamethicin molecules were observed to switch between two states: in one, the majority of the peptide molecules bind parallel to the membrane surface; in another, the majority of the peptide molecules insert perpendicularly into the membrane. The state of alamethicin was monitored by the method of oriented circular dichroism (OCD; Wu, Y., H. W. Huang, and G. A. Olah, 1990, *Biophys. J.* 57:797–806) using aligned multilayer samples in the liquid crystalline L_α phase. If L/P exceeds a critical value, most of the peptide molecules are on the membrane surface. If L/P is below the critical value, most of the peptide molecules are incorporated in the membrane when μ is high; when μ is low, most of them are again on the membrane surface. In a typical conduction experiment of voltage dependence, alamethicin molecules are in a partition equilibrium between the aqueous phase and the lipid phase before the application of voltage; in the lipid phase, the lipid/peptide ratio is such that most of alamethicin molecules are on the membrane surface. This is the nonconducting state of alamethicin. The OCD analysis showed that there is essentially no change in the secondary structure when alamethicin changes between the surface state and the inserted state. The voltage-gating mechanism can be explained if we assume that these surface peptide molecules probabilistically turn into the membrane core to form channels due to the dipole-electric field interactions. We speculate that the phase-transitionlike behavior is a manifestation of membrane-mediated intermolecular interactions between peptide molecules.

INTRODUCTION

Alamethicin, an antibiotic produced by the fungus *Trichoderma viride,* is a linear icosapeptide which spontaneously inserts into black lipid membranes and the membranes of some living cells producing a voltage-dependent ion conductance (Mueller and Rudin, 1968). It is perhaps the most extensively studied voltage-gated channel former (see references cited below). Recently, the alamethicin channel has also been shown to be tension dependent (Opsahl et al., 1990). Although the peptide consists of only 20 amino acids (Pandey et al., 1977), its channeling activity may be relevant to that of physiological channel proteins which typically consist of 2,000 or more amino acids. It is now generally thought that ion-conducting pores of proteins are formed by aggregates of transmembrane α-helical segments; these segments may be amphiphilic with the polar residues facing the pore and apolar residues on the other side (Guy, 1984; Finner-Moore and Stroud, 1984; Greenblatt et al., 1985; Lear et al., 1988). Alamethicin forms an amphiphilic helix in crystals, with its polar residues lying along a strip parallel to the axis (Fox and Richards, 1982). In membrane-associated states, perhaps 60–70% of alamethicin residues are in the α-helical form (see the experimental section). Presumably the alamethicin channel is a pore formed by an aggregate of such partial helices, similar to the pores in channel proteins. Thus it is of interest to study alamethicin as a simple model of voltage-gated as well as tension-sensitive ion channels. Its change of state with voltage may also illuminate the nature of protein-lipid interactions in general.

The assumption that alamethicin monomers form a water-filled conducting pore like the staves of a barrel is supported by most kinetic data of ion conductions (Latorre and Alvarez, 1981). However, the conducting state represents only the end state of a voltage-dependent process. To understand the voltage-gating mechanism, one also needs to know the initial state, and the intermediate states, if any. Conduction experiments provided no clues for the nonconduction state, either about the location of the molecule (relative to the membrane) or its configuration. Given its chemical structure, four plausible states have been suggested for alamethicin in the absence of an applied voltage, i.e., dispersed in the aqueous solution, bound parallel to the lipid-water interface, incorporated in the bilayer, or partly incorporated in the bilayer (presumably the α-he-

lical part) and the rest either projecting into the solution or bound to the interface. In each of these states, the peptide may be either monomers or aggregates. Thus, theoretically there are many alternative pathways that alamethicin might take in response to an applied electric field, and each may, in principle, satisfy the voltage dependence of the measured macroscopic conductance (see background section). In the last ten years, numerous spectroscopic and other methods have been used to study the nonconducting state, including Raman spectroscopy (Lis et al., 1976; Banerjee et al., 1985; Knoll, 1986), ^{1}H, ^{2}H and ^{31}P nuclear magnetic resonance (NMR) studies (Banerjee et al., 1983; Banerjee et al., 1985), infrared attenuated total reflection spectroscopy (Fringeli and Fringeli, 1979), alamethicin-phospholipid cross-linking studies (Latorre et al., 1981), titration and stopped-flow analyses using circular dichroism (CD) and fluorescence to monitor the alamethicin-lipid interactions (Schwarz et al., 1986; Schwarz et al., 1987; Rizzo et al., 1987), capacitance studies (Vodyanoy et al., 1988), and studies of synthetic analogues (Vodyanoy et al., 1982; Hall et al., 1984; Menestrina et al., 1986). The results have led to many different conclusions. In particular, there are conflicting interpretations as to whether, in the absence of a transmembrane electric field, alamethicin partitions into the apolar region of a lipid bilayer or adsorbs to the lipid-water interface. Lis et al. (1976), Banerjee et al. (1983), Banerjee et al. (1985) and Vodyanoy et al. (1988) found evidence for interfacial interactions. But Fringeli and Fringeli (1979), Latorre et al. (1981), Knoll (1986), Schwarz et al. (1986), Schwarz et al. (1987) and Rizzo et al. (1987) concluded that alamethicin inserts into bilayers.

In this paper we report the discovery of a new phenomenon of alamethicin-membrane interactions. We discovered a phase-transitionlike behavior between a state in which the majority of alamethicin molecules bind parallel to the membrane surface and a state in which the majority of alamethicin molecules insert perpendicularly into the membrane, as a function of lipid/peptide ratio and of the chemical potential of water. The state of alamethicin was monitored by the newly developed method of oriented circular dichroism (OCD; Wu et al., 1990) using aligned multilayer samples in the liquid crystalline L_α (smectic A) phase (no lipid phase transitions are involved). If the lipid/peptide ratio exceeds a critical value, the majority of alamethicin molecules are on the membrane surface. If the lipid/peptide ratio is below the critical value, the majority of alamethicin molecules are incorporated in the membrane when the degree of hydration is high; when the degree of hydration is low, the majority of alamethicin molecules are again on the membrane surface. In a typical conduction experiment, alamethicin molecules are in a partition equilibrium between the aqueous phase and the lipid phase; and in the lipid phase, the lipid/peptide ratio is such that most of the alamethicin molecules are on the membrane surface in the absence of a field; this is the nonconduction state of alamethicin. As we will show, the state of alamethicin also depends on membrane composition (it may also depend on the experimental procedure if the sample is measured before it reaches equilibrium); this may explain why different states of alamethicin were observed in different experiments as reported in the literature.

The phase-transitionlike behavior seems to be a cooperative phenomenon; that is, the states of the majority of peptide molecules are correlated in changing from one to another. When alamethicin is incorporated in membrane (perhaps in aggregate forms) the hydrophobic regions of lipid and peptide will tend to match each other; and this matching may cause some deformation in the configuration of the bilayer. The range of such a deformation can indeed be long (many nanometers; see Huang, 1986); and when two deformation regions overlap, the deformation free energy is lowered. Such membrane-mediated intermolecular interactions between protein molecules have been discussed before (Marcelja, 1976; Schröder, 1977), in particular by Pearson et al. (1984) who used a general (or nonspecific) free energy for membrane deviated from equilibrium, and by Huang (1986) who used the elastic free energy for membrane deformation. We speculate that membrane-mediated interactions between alamethicin molecules may explain the phase transitionlike behavior we observed.

BACKGROUND

The ion conduction properties of alamethicin, in the single- and multichannel modes, were comprehensively characterized in the 1970's, notably by Eisenberg et al. (1973), Gordon and Haydon (1975), Boheim and Kolb (1978); the subject was succinctly reviewed by Latorre and Alvarez (1981).

Based on the conduction data, Bauman and Mueller (1974) and Boheim (1974) were the first to propose a molecular model for the alamethicin channel. In it they assume that the channel consists of a nucleus that grows in diameter through the uptake of monomers; and alamethicin has a dipole moment so that the voltage-dependent step is the rotation of alamethicin monomers by the electric field from the surface into the membrane. The model is represented by the pathway $1 \rightarrow 2 \rightarrow 3$ in Fig. 1 in which $2 \rightarrow 3$ is the voltage driven step (Latorre and Alvarez, 1981). The dipole moment of alamethicin molecule can be estimated from the voltage dependence

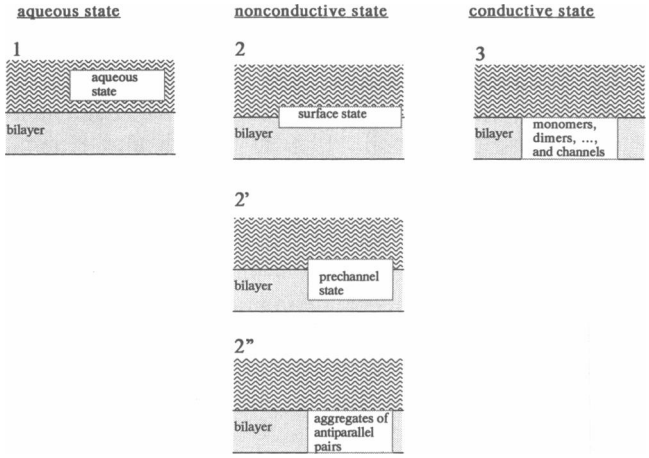

FIGURE 1 Possible states of alamethicin in a conduction experiment. 1: Dissolved in the aqueous solution. 2: Bound parallel to the lipid-water interface. 2′: A prechannel is a conjectured configuration in which the α-helical parts of a number of monomers aggregate like a channel but the nonhelical parts of the monomers either projecting into the solution or bound to the interface as proposed by Boheim and Kolb (1978), Fox and Richards (1982), and Hall et al. (1984). 2″: Aggregates of dimer pairs of antiparallel-arranged helix dipoles as proposed in the dipole flip-flop model (Boheim et al., 1983; Menestrina et al., 1986). 3: Peptide molecules incorporated in the bilayer exist in various forms of aggregate including monomers, dimers, et cetera, as well as the channel forms.

of the alamethicin-induced steady state macroscopic conductance. For example, in dioleoylphosphatidylcholine (DOPC)-decane membrane the conductance is approximately proportional to $\exp(6e\psi/k_BT)$, where e is the electronic charge; ψ is the applied voltage across the membrane; k_B is the Boltzmann constant; and T is the absolute temperature (Latorre and Alvarez, 1981). Assuming that the channel length is ~30 Å and each channel is formed by 10 monomers (this is deduced from the experimental evidence that the conductance is roughly proportional to the 9th power of alamethicin concentration in solution), one obtains 18 eÅ for the dipole moment per monomer (1 eÅ = 4.803 D). This is in reasonable agreement with the value measured in lipophilic solvents, ~15 eÅ (Schwarz and Savko, 1982; Yantorno et al., 1982), or with 14 eÅ estimated for a straight helix of 20 amino acids (Olah and Huang, 1988*b*). Variations of this original model were also proposed, mainly concerning the possibility of some forms of alamethicin aggregate on the membrane surface, micelles (Hall, 1975) or prechannels (Boheim and Kolb, 1978).

More drastic revisions were proposed after Fringeli and Fringeli (1979) and Latorre et al. (1981) found alamethicin incorporated into the hydrophobic core of the membrane in the absence of an applied field. All the subsequently proposed models bypass the surface state. Fox and Richards (1982) and Hall et al. (1984) proposed specific prechannels for the nonconducting state, in which the α-helical part of alamethicin already inserts into the membrane in the absence of an applied field, corresponding to the pathway 1 → 2′ → 3 in Fig. 1. Boheim and his colleagues (Boheim et al., 1983; Menestrina et al., 1986) proposed a dipole flip-flop model, in which a channel is formed out of a preexisting dodecamer aggregate of six dimer pairs of antiparallel-arranged helix dipoles; the application of a sufficiently strong electric field would force all helices to assemble in a parallel array; the model is represented by the pathway 1 → 2″ → 3 in Fig. 1. Most recently, Schwarz and his colleagues (Schwarz et al., 1986; Schwarz et al., 1987; Rizzo et al., 1987) proposed the pathway 1 → 3 (Fig. 1) to account for the voltage gating of alamethicin channels.

EXPERIMENT

Diphytanoylphosphatidylcholine (DPhPC) in $CHCl_3$ (20 mg/ml) was purchased from Avanti Polar Lipid Inc. (Pelham, AL). It was diluted to give a stock solution of 10 mg/ml. Alamethicin was purchased from Sigma Chemical Co. (St. Louis, MO) and was used without further purification. A stock solution of alamethicin was prepared in $CHCl_3$:CH_3OH (1:1) at a concentration of 1 mg/ml, and stored at −20°C. For each sample batch, 10 ml of DPhPC stock solution was mixed with an appropriate amount of alamethicin stock solution at the desired lipid/peptide molar ratio (L/P). A clean nitrogen stream was blown on the solution so as to remove the solvent and then it was further dried under vacuum (5 μm) for 4 h. Approximately 10 ml of distilled water was added to the dry mixture. The mixture was then homogenized so as to break up any large aggregates. The lipid/alamethicin dispersion was sonicated, lyophilized and left under vacuum for 24 h. The fluffy powder was removed from vacuum and placed in a container, which was in turn placed in a larger flask containing a small amount of water. The flask was then sealed and stored in the dark. The top of the sample container was left open so the sample would be in contact with H_2O vapor. The sample was incubated for three to five days at high humidity and room temperature (~22°C) until it appeared to be a clear gel.

A small amount from each sample batch was then sandwiched between two fused silica plates (1 × 1 in × 0.5 mm) without a spacer. Each sample was aligned homeotropically (lipid bilayers parallel to silica surfaces) by the procedure described in Huang and Olah (1987). A Bragg reflection pattern obtained by the method of θ–2θ scan off such a sample (aligned between

a polished beryllium plate and a silica plate) is shown in Fig. 2, indicating a well ordered, layered structure. Our analysis (the method has been described in Olah et al., 1991 and Liu et al., 1991) shows that the phosphate-to-phosphate distance across the bilayer is ~34 Å. The lamellar spacing for the sample equilibrated with 100% relative humidity is ~48 Å, indicating a water layer of ~14 Å in thickness between lipid bilayers. The thickness of the water layer decreases by 5 Å if the relative humidity is reduced to 50%.

Hydrated DPhPC/alamethicin multilayer samples at 22°C are in general in the L_α (smectic A liquid crystalline) phase, as determined by visual inspection between crossed polarizers with a microscope. The determination is possible because the defect structures of the L_α phase have been classified and studied by polarization microscopy; and its fluidity and texture are different from the gel phase (Asher and Pershan, 1979*a*,*b*; Schneider and Webb, 1984; Huang and Olah, 1987). If a sample changes into the gel phase, as it may happen when a sample with a high peptide concentration is exposed to a very low humidity, it is readily recognizable by its birefringence and change of texture.

A series of sealed jars containing saturated solutions of various salts were used as humidity chambers (O'Brien, 1948). The aligned multilayer samples were placed in the humidity chambers to be equilibrated at chosen relative humidities (RH) in room temperature. The sample obviously exchanged water with its environment through the gap between the two silica plates. Depending on its initial and final RH, or more precisely, its initial and final positions on the phase diagram (see below), the equilibration time for a sample varies from 4 to 20 days. Throughout the ranges of L/P and RH to be reported below, except where noted otherwise, the samples were observed to be in the L_α phase.

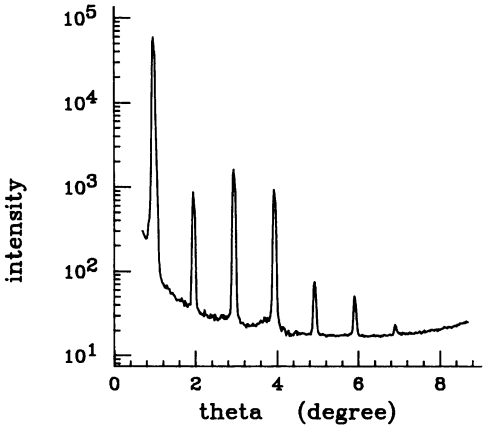

FIGURE 2 An x-ray reflection pattern of an aligned alamethicin/DPhPC (molar ratio 1/140) multilayer sample obtained by θ–2θ scan. The lamellar spacing for this pattern is 45.1 Å.

CD of alamethicin associated with DPhPC and dilauroylphosphatidylcholine (DLPC) vesicles have been measured before (Wu et al., 1990 and Olah and Huang, 1988*a*, respectively). If we assume that the mean residue ellipticity of α-helices is independent of the length of peptide and compare the CD of alamethicin with that of a standard α-helix (e.g., poly-γ-methyl-L-glutamate in hexafluoroisopropanol; see Olah and Huang, 1988*b*), we would conclude that ~40% of the residues of alamethicin are helical. However, there is evidence for the length effect; for example, synthetic α-helical peptides of 21 amino acids show a mean residue ellipticity of $\sim -2 \times 10^4$ deg cm^2 decimol^{-1} at 224 nm (Degrado and Lear, 1990) which is only ~60% of the standard value for long α helices. Therefore, in fact as much as 60–70% of alamethicin residues could be α helical.

The method of oriented circular dichroism (OCD) was described in detail in Wu et al. (1990). In essence, CD of an aligned multilayer sample was measured with light incident at the normal as well as oblique angles with respect to the plane of the membranes. This allows us to unambiguously determine the orientation of the α-helical section of alamethicin relative to the plane of membrane. Two sets of OCD were shown in Fig. 6 of Wu et al. (1990): spectra *A*, *B*, *C* (from a sample of L/P = 50/1 at 100% RH) will be collectively called spectra *I*; and spectra *D*, *E*, *F* (from the same sample at 50% RH) collectively called spectra *S*. It was shown in Wu et al. (1990) that the orientational dependence of spectra *I* describes helices perpendicular to the plane of the membrane; we call this the inserted or incorporated (*I*) state; on the other hand, the orientational dependence of spectra *S* describes helices parallel to the plane of the membrane; we call this the surface (*S*) state.

It is clear from the OCD analysis that spectra *I* and spectra *S* are directly related by rotation; therefore, the secondary structure of alamethicin in these two states are essentially the same. Furthermore, one could use either spectra *I* or spectra *S* and mathematically generate a spectrum for randomly oriented alamethicin, and it had been shown to be the same as the measured CD of alamethicin in vesicles (Wu et al., 1990). (Thus, it is impossible to use vesicular CD to determine whether alamethicin is on the membrane surface or incorporated in the membrane.)

The equilibrium states of alamethicin in DPhPC at various values of RH and L/P were studied by the method of OCD. The result shown in Fig. 3 resembles a phase diagram. Disregarding the region near L/P = 10/1 and RH ≤ 89% where the sample is in the gel phase (*shaded area* in Fig. 3), the phase diagram has three distinct regions. In the region of open circles,

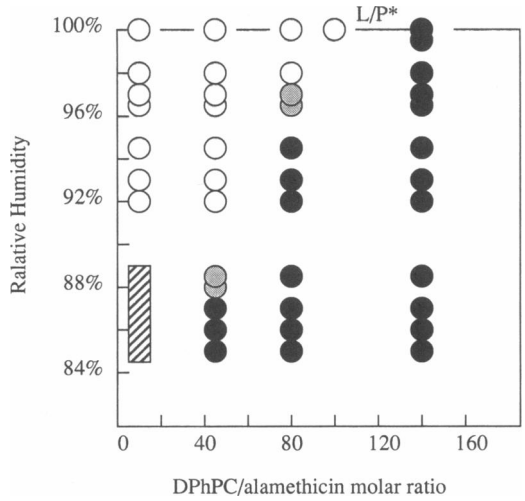

FIGURE 3 The phase diagram for alamethicin in DPhPC on the plane of relative humidity (RH) versus the lipid/peptide molar ratio (L/P). A multilayer sample of a certain L/P was in turn equilibrated in humidity chambers of various RH; in each equilibrium state, its OCD was measured. If the OCD is close to spectra I, indicating that the majority of alamethicin molecules are in the inserted state, an open circle is shown at the corresponding L/P and RH. If the OCD is close to spectra S, indicating that the majority of alamethicin molecules are in the surface state, a black circle is shown. A gray circle implies that the OCD is a linear superposition of comparable portions of spectra I and spectra S. The shaded area for L/P = 10/1 indicates that the sample at RH < 89% turned into the gel phase. In all other data points, the samples were in the L_α phase. There is a critical value of L/P, denoted as L/P*. For L/P > L/P*, the majority of alamethicin molecules are clearly in the surface state; for L/P < L/P*, the majority of alamethicin molecules are in the inserted state if the sample is in equilibrium with 100% RH.

OCD indicate that >80% of alamethicin molecules are in the inserted state; in the region of black circles, >80% of alamethicin molecules are in the surface state; in between them where the gray circles are shown, comparable amounts of peptide molecules are in both states. In each region the OCD spectra at different data points are similar but not exactly the same. For example, in the region of open circles, the spectra of the data points which are far away from the gray region are the same as spectrum *I*; as the points get closer to the gray region, the spectrum seems to contain an increasing fraction of spectrum *S*. The converse is true in the region of black circles. In the region of gray circles, the spectra are roughly in the middle between spectrum *I* and spectrum *S*. For convenience, we will call the region of open circles the *I* region, the region of black circles the *S* region and the region of gray circles the transition region.

The reversibility of the hydration/dehydration processes was examined by exchanging samples of same L/P between two humidity chambers; the spectra were always exchanged. If the samples were exchanged again, the original spectra were reproduced. The data points near the transition region were reproduced by two or three different samples. When a sample was moved to a different humidity chamber, it took a longer time (up to 20 d) to reach equilibrium, if the final RH was closer to the transition region. An equilibrium spectrum remained unchanged in time as long as the sample was kept in the same humidity chamber. The samples were examined microscopically between crossed polarizers each time a CD measurement was made to ensure that the multilayers were in the L_α phase. We note that samples of L/P = 10/1 change to the gel phase at RH ≤ 89% (*shaded area,* Fig. 3).

Dioleoylphosphatidylcholine (DOPC) and L-α-phosphatidylcholine from bovine brain (BBPC) were purchased from Sigma Chemical Co. They were used like DPhPC. Alamethicin in BBPC at L/P ~ 50/1 showed an I ↔ S transition as RH was varied. The result of alamethicin in DOPC is shown in Fig. 4, using the symbols of Fig. 3. Fig. 5 shows an example of spectral changes during transition for alamethicin in DOPC. Again, the samples were inspected to be in the L_α phase throughout the observed region of the phase diagram Fig. 4.

SUMMARY OF EXPERIMENT AND DISCUSSIONS

It should be stressed that the transitions between the surface state and the inserted state of alamethicin observed in this experiment are not related to the lipid phase changes. Indeed, alamethicin in ditetradecylphos-

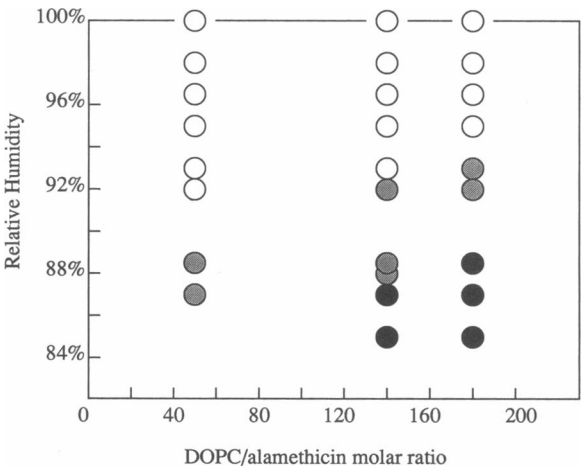

FIGURE 4 The phase diagram of alamethicin in DOPC. See Fig. 3 for explanations.

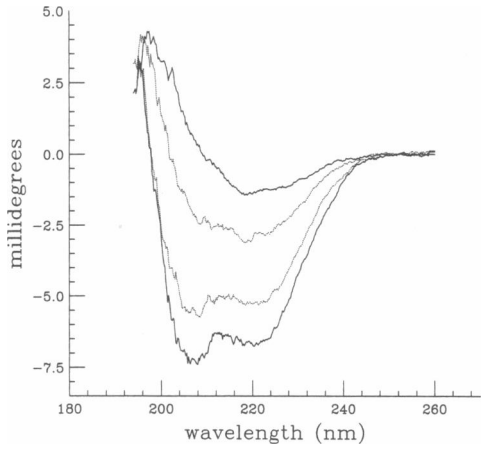

FIGURE 5 OCD of a multilayer sample of alamethicin in DOPC with L/P = 50/1 measured at the normal incidence $\alpha = 0°$ (not normalized). The top spectrum was obtained when the sample was initially in equilibrium at 100% RH. The sample was then placed in a humidity chamber of 50% RH. The spectrum changed gradually from the top curve to the bottom curve; the latter is the equilibrium spectrum at 50% RH.

phatidylcholine has been observed to change from the inserted state to the surface state as the lipid changes from the L_α phase at 35°C to the gel phase at 15°C (Vogel, 1987); but this is altogether a different phenomenon.

The question about the position of the *S* state peptide molecules in the multilayer array is currently under investigation with x-ray and neutron scatterings. Nevertheless, we have evidence to show that alamethicin molecules in the *S* state are associated with membrane bilayers, rather than dissolved in the water layers. First, Rizzo et al. (1987) have shown that the CD spectrum of alamethicin in aqueous solution is substantially altered when lipid vesicles are added to the solution, indicating that the conformation of membrane-associated alamethicin is significantly different from that of dissolved peptide. Second, we have shown by OCD that the conformation of alamethicin is essentially the same in the *S* and *I* states; therefore, the *S* state is membrane-associated.

It is important to point out that the membrane-associated surface state is not unique to the multilayer configuration used in our experiment, where the water content between bilayers is low. Banerjee et al. (1985) have detected alamethicin associated with membrane surface in a membrane dispersion (1:10 w/v lipid to water). The capacitance measurement by Vodyanoy et al. (1988) was performed in an experimental setup similar to a conduction experiment; their result is consistent with alamethicin being adsorbed to the membrane surface under the condition of no conduction.

The phase diagram of alamethicin in DPhPC (Fig. 3) is characterized by a critical lipid/peptide molar ratio L/P* and a transition region. If the lipid/peptide ratio is $>$L/P* (that is, at a low peptide concentration), the majority of alamethicin molecules are always in the surface state in equilibrium. On the other hand, at a higher peptide concentration (L/P $<$ L/P*), the majority of alamethicin molecules are always in the inserted state when the system is in equilibrium at 100% RH. Starting from the critical L/P*, the transition region decreases to lower RH for lower L/P. On the high L/P side of the transition region, the majority phase in the surface state coexists with a minority phase in the inserted state. On the low L/P side, the majority phase in the inserted state coexists with a minority phase in the surface state.

The phase diagram of alamethicin in DOPC (Fig. 4), although it is incomplete, appears to have the same features. And we suspect that these features are the general characteristics of alamethicin in most lipid membranes. Different lipids apparently have different values of critical L/P*: ~140/1 for DPhPC; above 200/1 for DOPC. Because the sensitivity of CD spectrometers is limited to L/P ~ 200/1 to 300/1, it may be difficult to establish the phase diagrams for some lipids by the method of CD.

It is not surprising then that earlier studies produced conflicting interpretations on the state of alamethicin (whether it is on the surface or is inserted in the lipid core in the absence of voltage). We believe that it is the result of different investigators using different lipids, different lipid/peptide ratios and different preparation procedures.

A typical conduction experiment of voltage dependence is performed with a low peptide concentration associated with the membrane. In that case, the majority of peptide molecules will be in the surface state. This is the nonconduction state of alamethicin. The transition between the surface state and inserted state observed in our experiment implies that there is a pathway between these two states with no change in the secondary structure. When a transmembrane voltage is applied, the probability for the surface alamethicin to turn into the inserted state is increased by a Boltzmann factor due to the dipole-electric field interaction. This is precisely the original Bauman-Mueller-Boheim model (Bauman and Mueller, 1974; Boheim, 1974).

THEORY OF PHASE TRANSITION

There seems to be no obvious explanation for the alamethicin-bilayer interactions observed in this experiment. For example, the insertion of alamethicin into

membrane can not be explained as due to the lack of water between bilayers; the hydration dependence is contrary to this argument. Nor can it be understood as the peptide solubility to lipid; if it were so, one would expect a larger fraction of alamethicin molecules inserted at a higher L/P. Perhaps, the most unexpected result is the abrupt change of state for the majority of alamethicin molecules across the transition region. It is difficult to see how this can happen if the peptide molecules are not all directly or indirectly interacting with each other.

During our experiment, we did not notice any aggregation effect in the samples: within the entire sample area (25 × 25 mm) the multilayers appeared to be uniform under microscope; essentially the same CD spectrum was produced in any region of the sample with a light beam of 3 × 4 mm in cross-section; the transitions between the inserted state and the surface state were reversible and reproducible. If the peptide molecules, say at a peptide/lipid molar ratio of 1/100, are uniformly distributed in the membrane either in the monomeric form or in the forms of small aggregates, how do they interact with each other to produce long-range correlations? In the following we offer a speculative answer to this question.

Because alamethicin is amphiphilic, it is likely to form aggregates in the membrane. Furthermore, the hydrophobic regions of peptide and lipid will tend to match each other because the hydrophobic-hydrophilic mismatch is energy costly, ~24 cal per Å^2 of accessible surface area of proteins (for details see Chothia, 1974). However, lipid bilayers are more deformable than proteins, therefore the bilayer is likely to be deformed from its protein-free equilibrium configuration to accommodate an inserted protein. (For example, the thickness compressibility of a lipid bilayer is ~400 times the volume compressibility of proteins such as myoglobin and lysozyme [see Huang, 1986; Frauenfelder and Marden, 1981]). In a previous paper considering the membrane thickness effect on the gramicidin channel lifetime, one of us (Huang, 1986) has shown that the range of membrane deformation due to a protein insertion is of the order of $2(DK_c/\overline{B})^{1/4} \sim 32$ Å, where D ~ 35 Å is the thickness (Olah et al., 1990), $K_c \sim 10^{-12}$ erg the curvature elastic modulus (Schneider et al., 1984; Duwe et al., 1990; Mutz and Helfrich, 1990), and $1/\overline{B} \sim 2 \times 10^7$ Å^2/dyne the thickness compressibility (Hladky and Gruen, 1982) of the bilayer. Each inserted alamethicin aggregate is the center of a circular area of bilayer deformation. When two deformation areas overlap, the total free energy of deformation decreases; therefore, there is an interaction between the two inserted aggregates.

Let us consider a collection of alamethicin aggregates in the inserted state. They are designated by indices i = 1, 2, 3, et cetera. We also designate the state of the ith aggregate by a symbol σ_i. Let $\sigma_i = 0$ if the aggregate changes into the surface state, otherwise $\sigma_i = 1$. The energy of the system can be written as

$$H = -\sum_{(ij)} G_{ij}\sigma_i\sigma_j + \sum_i f_i\sigma_i, \tag{1}$$

where $-G_{ij} < 0$ represents the interaction energy between the ith and the jth aggregates if their areas of bilayer deformation overlap; (ij) represents pairs of interacting aggregates; f_i represents the free energy difference of the ith aggregate between the inserted state and the surface state, provided there are no other peptide molecules present in the system. Because we are only interested in the qualitative nature of such a system, we shall assume that all aggregates have the same number of monomers, each aggregate interacts with η neighboring aggregates, all G_{ij}'s have the average value G, and all f_i's have the same average value f. The energy of the system is then simplified to

$$H = -G\sum_{(ij)} \sigma_i\sigma_j + f\sum_i \sigma_i. \tag{2}$$

This model can be shown to have a first-order phase transition between the $\langle\sigma_i\rangle \sim 0$ state and the $\langle\sigma_i\rangle \sim 1$ state, where $\langle\ \rangle$ represents the ensemble average (Huang, 1975). The phase boundary is given by

$$f = \eta G/2. \tag{3}$$

Fig. 6 shows a schematic phase diagram for the model described by Eq. 2. Because high relative humidity favors the inserted state, we expect f to be a decreasing function of RH. Also we expect the interaction G to increase with the peptide concentration, that is, G is a decreasing function of L/P. Thus the phase diagram Fig. 6 has the similar characteristics of the phase dia-

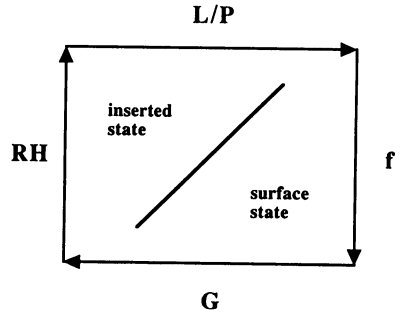

FIGURE 6 The phase diagram for Eq. 2 in f versus G. The direction of increasing RH is indicated on the f axis; the expected direction of increasing L/P is indicated on the G axis. The phase diagram is qualitatively similar to Figs. 3 and 4.

grams shown in Figs. 3 and 4. Obviously, further studies are needed to understand this interesting cooperative phenomenon of peptide molecules in membrane.

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