Membrane Specificities of Antimicrobial Peptides

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Abstract

Antimicrobial peptides target the lipid matrix of plasma membranes. Their biological functions can be understood in terms of the free energy of peptide-membrane interactions.

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During the past decade, endogenous antimicrobial peptides have become recognized as important, ubiquitous, and ancient contributors to the innate mechanisms which permit animals (including humans) and plants to resist infection (Boman et al., 1994). Most of these host defense peptides are small (18-35 amino acids), amphipathic and possess either an α-helical or cystine-stabilized β-sheet structure. They are most likely too small for enzymatic function and, so far, no specific receptors have been found. All evidence indicates that their target of action is the lipid matrix of the plasma membranes. What are the molecular mechanisms of these peptides? How do they kill bacteria without harming the host cells? Indeed the most interesting question is: how do they accomplish the membrane specificities? These questions provide a good test to our current understanding of membrane properties.

In this review article, we will concentrate on helical peptides. In particular, we will discuss the experimental data of alamethicin (a fungal peptide) and magainin (a peptide secreted in frog skin). Magainin exhibits a broad-spectrum of antibacterial, antifungal, and tumoricidal activities. However, at the bactericidal concentrations, the peptide does not harm eukaryotic cells–it does so only if the concentrations are increased 100- to 1000-fold. On the other hand, alamethicin is hemolytic at the concentrations it is bactericidal. However, this does not mean that alamethicin lyses all cells equally. Even among various bacteria, there is a 1000-fold difference in their sensitivity to alamethicin (Jen et al., 1987).

In their natural environment, antimicrobial peptides interact with cell membranes in two steps.
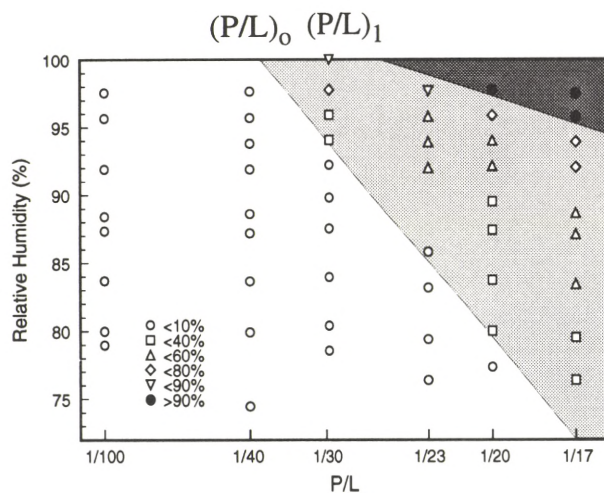
Figure 1. Orientation of alamethicin helices in DPhPC bilayers in the phase diagram P/L vs relative humidity. The symbols represent the percentage of alamethicin oriented perpendicularly.

First they bind to the lipid matrix of the membranes. This step has been studied by vesicle binding experiments. As expected, positively charged peptides preferentially bind to acidic lipids (which are present on the outer surface of bacterial plasma membranes). However, if the binding of peptides were enough to kill cells, this electrostatic effect alone does not provide a good selectivity. Why? Because these peptides are amphipathic and they do bind to neutral lipid membranes. Besides, there are examples countering the electrostatic argument: for instance, melittin is more effective in lysing neutral liposomes than acidic liposomes. We have to examine the second step, i.e., what happens after the peptides bind to the membranes.

Matsuzaki et al. (1995) showed that once a peptide binds to a bilayer surface, it quickly redistributes itself to both sides of the bilayer by translocation. Therefore it is appropriate to consider peptide-membrane interactions with the peptides bound to both sides of the membrane. Our experiments started with a peptide-lipid mixture at a molar ratio (P/L). The mixture was aligned into parallel lamellae. The water content of the sample was determined by the relative humidity which the sample was equilibrated with. Since the sample tends to flow off the substrate if the relative humidity is $\geq$ 98%, the experimental range of the

relative humidity was ~70% to ~98% RH (most experiments were performed in the L_α phase). The hydration dependence of our experiment is extremely helpful for interpreting the results. In particular we are able to extrapolate the results to the full hydration from the humidity dependent data.

First we measured the orientation of the helical axes of the peptides relative to the plane of the lipid bilayers. This was conveniently accomplished by the method of oriented circular dichroism (Wu et al., 1990). Fig. 1 shows the orientation of alamethicin helices in diphytanoylphosphatidylcholine (DPhPC) bilayers at various P/L and RH. Most surprisingly, the result resembles a phase diagram. For P/L below a critical value, $(P/L)_0$ ~1/40, the helical peptides adsorb parallel to the membrane surface. Above the critical concentration, however, a fraction of the peptides are oriented perpendicular to the bilayers while the rest remain on the membrane surface. Above an even higher concentration, $(P/L)_1$, all of the peptides are oriented perpendicular to the plane of the membranes. It appears that this is the general behavior of alamethicin in all lipid bilayers (Huang and Wu, 1991). However, in most lipids, the critical concentration $(P/L)_0$ as well as $(P/L)_1$ are very low, so that within the experimental range of P/L where the peptide orientation is detectable,

Barrel-stave model   Toroidal (wormhole) model
(alamethicin)   (magainin)
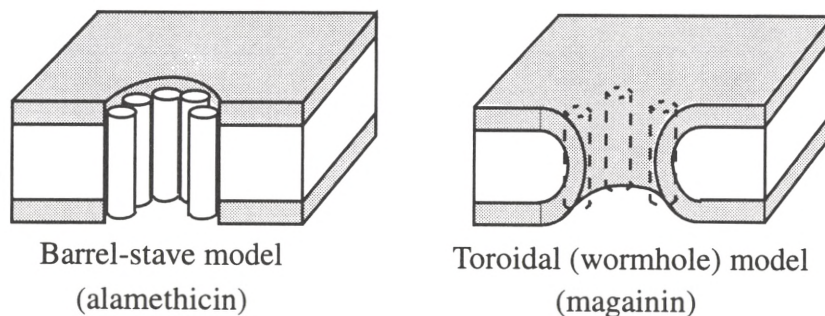
Figure 2. Alamethicin inserts into a lipid bilayer by forming a cylindrical pore with the peptide monomers lining the periphery. (The small cylinders represent the helical peptide monomers. The shaded area represents the headgroup region of the lipid bilayer. The dotted cylinders represent the peptide monomers embedded in the headgroup region.) Magainin always associates with the headgroups of the lipid. When magainins insert, they carry the headgroups with them. So the top monolayer bends and merges with the bottom monolayer like the inside of a torus.

one sees only the phase of perpendicular orientation (e.g., in DMPC, POPC, etc.). Magainin also exhibits a similar phase diagram, but whether it has an all-perpendicular concentration $(P/L)_1$ is not clear (Ludtke et al., 1994).

The samples with $P/L>(P/L)_0$ were examined by neutron scattering (He et al., 1995; 1996a; Ludtke et al., 1996). We found pores in the membranes whenever there are peptides perpendicularly oriented. Interestingly alamethicin and magainin form two different types of pore (Fig. 2). Both pores are large (water pathways ≥ 20 Å in diameter). A high density of pores in the cell membrane is apparently lethal to the cell. On the other hand, below the critical concentration, $P/L<(P/L)_0$, the great majority of the peptide are adsorbed on the surface. Through thermal fluctuations, a small number of transient pores (lifetimes being ms) may appear as detected by patch-clamp measurements. Because cells have repair mechanisms, these transient pores are presumably nonlethal (Boman et al., 1994). Therefore, what determines the action of a peptide is its membrane-bound concentration relative to the critical concentration $(P/L)_0$. To see the significance of the phase transition, let us imagine that the pore state and the surface state are two states of the peptide with different energies. Without cooperativity, the fraction of the peptide forming pores is determined by a Boltzmann factor, independent of the pep-

tide concentration. With a phase transition, the system gains a control parameter, i.e., the concentration. And there can be a range of concentration in which the peptide is a very effective antimicrobial (Fig. 3).

To see what causes the phase transition, we have to examine the energetics of the peptide-membrane interactions beyond the principle of hydrophobic matching. The clue comes from x-ray diffraction experiments. We found that the membrane thickness decreases linearly with the peptide concentration on its surface (Fig. 4). This is the evidence that the peptide is adsorbed within the headgroup region of the bilayer. The adsorption expands the bilayer laterally and hence reduces its thickness (Fig. 5). From the (fractional) decrease in the thickness, one obtains the (fractional) increase in the lipid area $\Delta A/A_0$ as a function of P/L. We found $\Delta A(L/P)$ equal to the cross section of alamethicin lying parallel to the bilayer (Wu et al., 1995). Magainin also causes membrane thinning in the same manner (Ludtke et al., 1995). I proposed to describe the bilayer deformation energy F (per unit area) by (Huang, 1986; 1995)

$$F = aB\left[\frac{D(x,y)}{2a}\right]^2 + \frac{K_c}{8}[\Delta D(x,y)]^2$$
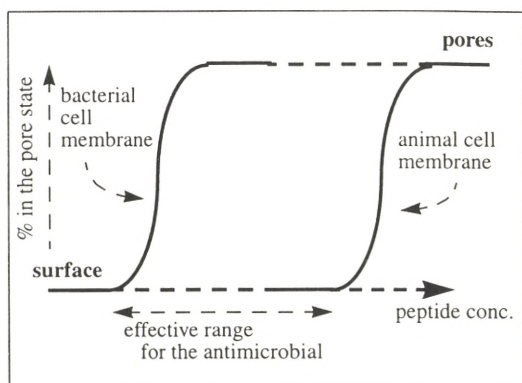
$$+\frac{K_c}{2}[\Delta M(x,y) - C_0(x,y)]^2. \tag{1}$$

Fig. 3
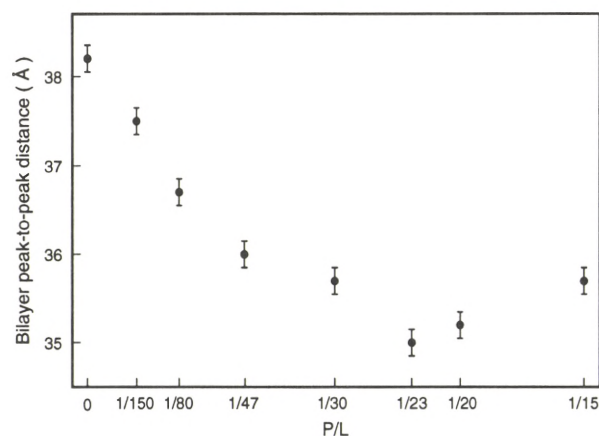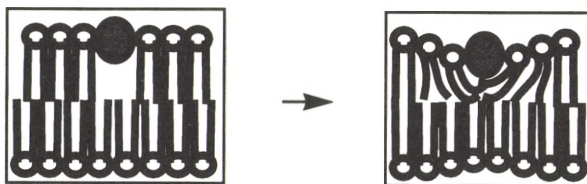**Fig. 4**

Figure 3. Significance of phase transitions.

Figure 4. The bilayer thickness is defined as the peak-to-peak (approximately phosphate-to-phosphate) distance in the electron density profiles measured by x-ray diffraction. For P/L<(P/L)$_0$, the thickness decreases linearly with alamethicin concentration.

Figure 5. Membrane thinning effect. Imagine a peptide in the headgroup region creating a gap in the chain region. For this gap to be filled, the membrane must become locally thinner.

The unperturbed bilayer is assumed to lie in the xy plane. $D(x,y)$ is the deviation of the bilayer thickness from the equilibrium thickness $2a$ at the coordinate (x,y). $M(x,y)$ is the displacement of the mid-plane of the bilayer from its equilibrium position. Δ is the Laplacian. B is the compressibility modulus of the bilayer. K_c is Helfrich's bending rigidity for a bilayer (Helfrich, 1973). $C_0(x,y)$ is the local spontaneous curvature induced by peptide adsorption. Only the change of the bilayer thickness (the D-mode) will concern us here. The free energy of thickness deformation consists of only the first two terms, the compressibility term and the splay term. The effect of pep-

tides adsorbed on the membrane surface is as follows: The peptide-induced membrane deformation has a characteristic length $\lambda = (aK_c/2B)^{1/4} \sim 13$ Å. There is a membrane mediated interaction between two adsorbed peptide monomers described by the potential $V(x)$, where $x = r/\sqrt{2\lambda}$ and r is the distance between the two monomers (Fig. 6). The potential is repulsive up to about $r \sim 37$ Å. Therefore the peptide is dispersed on the bilayer surface as monomers. At low concentrations (the average inter-peptide distance >37 Å), the total membrane deformation energy is proportional to the peptide concentration. However, if the concentration is high such that the average inter-peptide
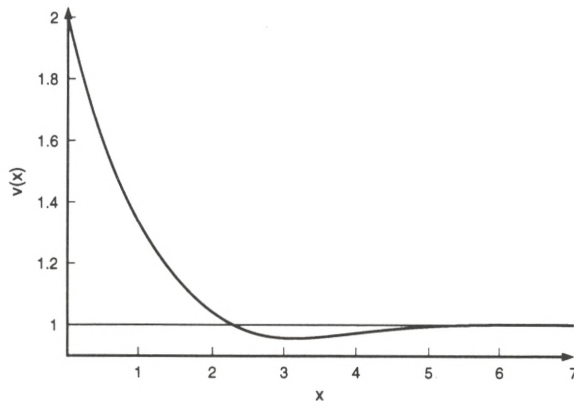
Figure 6. The membrane mediated interacting potential between two peptide monomers adsorbed on the surface, normalized to one at large distance. $x = r/\sqrt{2\lambda}$.

distance is <37 Å, the total deformation energy increases quadratically with the peptide concentration (Huang, 1995).

Thus the chemical potential μ_s of peptide adsorption consists of two parts: the binding energy (primarily due to hydrophobic matching) $-\varepsilon$ and the energy of membrane deformation per peptide f. As mentioned above, at high peptide concentrations f is proportional to P/L. Imagine that we gradually increase the peptide concentration from zero. At first μ_s is a constant. Then as the surface concentration exceeds $\sim (1/\sqrt{2\lambda})^2$, μ_s begins to increase linearly with P/L, until it becomes equal to the chemical potential for insertion μ_I. Until $\mu_s = \mu_I$, the great majority of the peptide molecules are adsorbed on the membrane surface. Only after μ_s reaches μ_I, a macroscopic insertion is possible. (In other words, as long as $\mu_s < \mu_I$, only a small number of transient pores appear in the membrane.) Thus the concentration that satisfies $\mu_s = \mu_I$ defines the critical concentration $(P/L)_0$. A mean-field calculation based on the free energy (1) showed that indeed a phase transition as described by Figs. 1 and 4 takes place (He et al., 1996b). In this model we showed that $(P/L)_0$ is decided by many factors including: the elastic constants of the bilayers, the binding energy difference between the surface state and the pore state, the area expansion of the bilayer per peptide

adsorbed and the thickness-matching condition of the bilayer to the inserted peptide.

Thus the specificity (or selectivity) of a peptide toward different cell membranes is determined by its binding coefficient and its critical concentration (of the bound peptide) for pore formation, $(P/L)_0$. Both are sensitive to the lipid composition of the membrane. The selectivities of these peptides are sharp (kill or harmless at a given concentration) because their actions are cooperative phenomena.

As a simple test to the above model, we predicted that if the size of the lipid headgroup is reduced, the bilayer will accommodate a higher concentration of peptide on its surface – in other words, the critical concentration $(P/L)_0$ will increase. By mixing a small amount of DPhPE into the pure DPhPC bilayer (Heller et al., 1997) the theory predicts:

$$\left(\frac{P}{L}\right)_0 (\theta) = \left(\frac{P}{L}\right)_0 (0) + \theta \left(\frac{\Sigma_{PC} - \Sigma_{PE}}{\Gamma}\right) \quad (2)$$

where θ is the fraction of PE in the PE-PC mixture. $(P/L)_0(\theta)$ is the critical concentration as a function of θ. Σ_{PC} and Σ_{PE} are the cross sections of PC and PE headgroup, respectively. Γ is the cross section of alamethicin. For example, the equation predicts that $(P/L)_0(0.1) \sim 1/25$ at
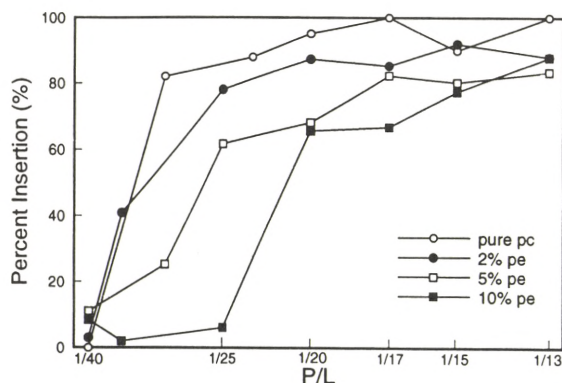
Figure 7. Percentage of alamethicin insertion as a function of P/L, measured by oriented circular dichroism, in pure PC and three PE-PC mixtures.

10%PE and $(P/L)_0(0.05) \sim 1/31$ at 5%PE. Both agree with the experiment quite well (see Fig. 7).

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