Molecular Mechanism of Peptide-Induced Pores in Membranes

Huey W. Huang*
Department of Physics & Astronomy, Rice University, Houston, Texas 77251, USA

Fang-Yu Chen and Ming-Tao Lee
Department of Physics, National Central University, Chung-Li, Taiwan 32054

(Received 22 August 2003; published 13 May 2004)

We suggest a physical mechanism by which antimicrobial peptides spontaneously induce stable pores in membranes. Peptide binding to a lipid bilayer causes an internal stress, or internal membrane tension, that can be sufficiently strong to create pores. Like detergents, peptides have a high affinity for the rim of the pore. Binding to the rims reduces the line tension and decreases the number of peptides causing the internal membrane tension. Consequently, the pore radius is stable. The pore formation resembles a phase transition.

DOI: 10.1103/PhysRevLett.92.198304 PACS numbers: 82.70.Uv, 01.55.+b, 05.70.Np, 87.16.Dg

In a recent series of studies on transient pores in stretched vesicles, Brochard-Wyart and collaborators [1–3] have demonstrated that the physics of pore formation in pure lipid bilayers is now well understood. The pore dynamics is based on the interplay between a membrane tension which tends to enlarge the pore and a line tension which tends to close the pore [4,5]. If the pore is on a vesicle, the process is coupled to the leakout of the internal fluid. By using viscous solutions, the time behaviors of pores were observed and analyzed in detail [1–3]. The purpose of this Letter is to suggest that the basic mechanism of pore formation can be extended to understand the action of antimicrobial peptides that are known to spontaneously induce pores in membranes. This pore-inducing phenomenon is related to the self-defense mechanism of plants and animals [6]. It can also be utilized to facilitate gene and drug delivery [6]. Although the spontaneous pore formation by antimicrobial peptides has been known for 30 years [7], the physics of this process has so far not been explained.

Naturally produced antimicrobial peptides are 20 to 40 amino acids long, and they assume a great variety of secondary conformations. Studies showed that the target of their functions is the lipid bilayers of cell membranes [6]. The fact that diverse antimicrobial peptides function in a similar way implies a common mechanism, and this mechanism cannot depend on the exact molecular structures [6,8]. One common molecular property of antimicrobial peptides is that the molecules are water soluble but the molecular surfaces are amphiphilic, i.e., detergentlike. Like detergents, they have great affinities for binding to lipid bilayers. Yet there is a so far unspecified characteristic difference between antimicrobial peptides and detergents, as the latter are not known to induce stable transmembrane pores.

In pure lipid bilayers pores are always produced under tension. The energy of a pore is given by $E_R^0 = 2\pi R\gamma - \pi R^2\sigma$ [4,5], where R is the radius of the circular pore, σ the membrane tension, and γ the line tension, or the free energy cost per unit length for creating the edge of the pore [2]. In most experiments, the process of pore opening was found to be stochastic and often a result of nucleation defects [3,9]. However, once a pore opens, its behavior is governed by γ and σ. For a radius greater than $R^* = \gamma/\sigma$, the pore expands indefinitely until the vesicle lyses. For a radius smaller than $R^* = \gamma/\sigma$, the pore closes. This general behavior of pores in pure lipid bilayers implies that antimicrobial peptides render two effects on membranes. The peptides must somehow create a stress equivalent to a membrane tension that induces pore formation and somehow stabilize the pores once produced. Here we propose the mechanisms for these two effects based on a long series of experiments published elsewhere.

By the virtue of amphiphilicity, a peptide is bound to a lipid bilayer at the interface between the hydrophilic headgroups and the hydrophobic chains. Normally, in a pure lipid bilayer, the hydrocarbon chains are closely packed on one side of the interface forming a hydrophobic barrier for the membrane, and the other side is packed with hydrated headgroups. A bound peptide inserts an additional area into the headgroup side of the interface (e.g., a 26 amino-acid peptide, melittin, adds 3 nm^2) [10], and thus causes a local deformation in the monolayer. Using the free energy of deformation (per unit area) $E_h = (K_a/2)(\delta h/h)^2 + (K_c/8)(\nabla^2 h)^2$ [11], where h is the hydrocarbon thickness, and K_a and K_c are, respectively, the stretch [12] and bending [13] moduli, we can estimate the range of deformation to be $\xi = (16h^2K_c/K_a)^{1/4}$ [14] (see Fig. 1). If the peptide area density is greater than $\xi^{-2} \sim 1/16$–$1/4$ nm^{-2} (depending on the values of the elastic constants), the local deformations by individual peptides overlap, and since the peptides do not penetrate the hydrocarbon region this region becomes thinner more or less uniformly due to the collective stretching by the peptides embedded in the headgroup region [10,14]. In

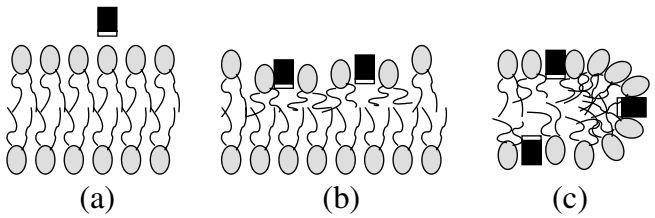

FIG. 1. Proposed interactions between peptides (rectangles) and lipid bilayers. The gray and black represent hydrophilic regions. (a) Peptides are soluble in water but have a high affinity for binding to lipid bilayers. (b) Peptides inserting into the headgroup region stretch the membrane area, cause thinning of the chain region, and thus create an internal membrane tension. (c) Peptides preferentially bind to the edges of the pores, which has the consequence of loosening the internal membrane tension. (The depicted pores are called toroidal, or wormhole models [18]. Some peptides make barrel-stave pores [20]. The mechanism should be the same for both types of pores.)

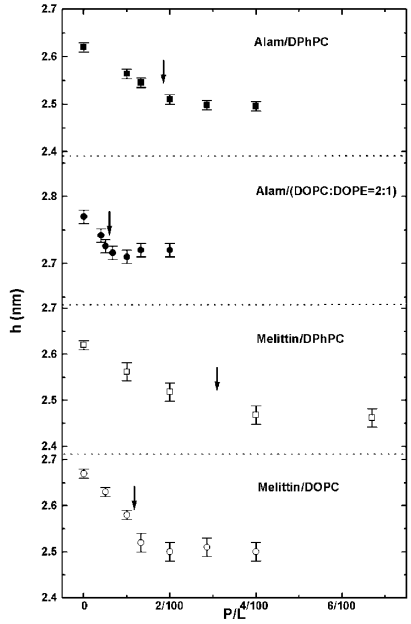

FIG. 2. Membrane thickness measured as a function of the peptide concentration P/L. Lipid vesicles with bound peptides were collapsed into parallel multilamella, from which x-ray diffraction measured the phosphate-to-phosphate distance (PtP) of the bilayer [10,16]. The hydrocarbon thickness h was obtained by subtracting twice the phosphate to chain distance ($\sim$1 nm) from PtP. Data of four different peptide/lipid systems are shown (from top): alamethicin in diphytanoyl phosphatidylcholine (DPhPC), alamethicin in a dioleoyl phosphatidylcholine (DOPC) and dioleoyl phosphatidylethanolamine mixture, melittin in DPhPC, and melittin in DOPC. (The data of alam/DPhPC and melittin/DOPC were reproduced from Ref. [10].) The arrows indicate the values for P/L^* as measured by oriented circular dichroism [10].

experiments, it was found that the bound peptides rapidly translocated from one monolayer to another via transient pore formation by fluctuations [15]. Thus one can assume that peptides are distributed on both sides of the bilayer. The membrane stretching and thinning caused by bound peptides have been measured in many peptide-lipid systems (see below).

Let the molar ratio of the bound peptide to lipid be P/L. The fractional change of the membrane area is $\delta A/A_L = (A_P/A_L)(P/L)$, where A_P is the area increment caused by one bound peptide and A_L the area per lipid. On the other hand, $\delta A/A_L = -\delta h/h$ and the latter is directly measurable by x-ray diffraction [16]. Figure 2 shows four examples of membrane thinning caused by peptide binding. Lipid vesicles with bound peptides were collapsed into parallel multilamella, from which the bilayer thickness, defined as the phosphate-to-phosphate distance, was measured by x-ray diffraction [10,16]. In each case, the membrane thickness decreases linearly with P/L initially and then reaches a plateau when P/L exceeds a critical value P/L^*. Two independent methods showed that transmembrane pores are formed in the membranes when $P/L > P/L^*$. First, oriented circular dichroism detected a change of peptide orientation and the change was proportional to $(P/L - P/L^*)/(P/L)$ [10,17]. Second, neutron in-plane scattering (with D_2O hydration) detected transmembrane pores in the membranes only if $P/L > P/L^*$ [18–20]. This description of peptide-membrane interactions is entirely consistent with the response of lipid vesicles exposed to melittin, one of the peptides reported in Fig. 2 [21]. Upon exposure to a low concentration of melittin, the lipid vesicle exhibited an area expansion (at constant volume) while no permeation through the membrane occurred. Fluorescence-labeled peptides were observed to bind to the vesicle concomitantly with the area expansion. At a high concentration of melittin, the membrane area first expanded rapidly at constant volume, then permeation occurred with a volume expansion, and finally the swelled vesicle lysed [21]. It is clear that peptide binding at low concentration caused an expansion of the membrane area without breaking the hydrophobic layer. When exposed to a high concentration of peptides, the membrane area also expanded without breaking the hydrophobic barrier until a high density of bound peptides caused permeation that was consistent with forming transmembrane pores.

The simplest explanation for the plateau of the membrane thinning is as follows: Assume that the peptides participating in the pore formation (P_I) do not contribute to the membrane thinning. Then, in the region $P/L > P/L^*$, the membrane area increase associated with thinning is $\delta A/A_L = (A_P/A_L)(P - P_I)/L$. This area expansion creates a stress equivalent to a membrane tension. We shall call this an internal membrane tension because it does not exert a force at the membrane boundary. However, its value should be similar to the external tension required to stretch the membrane area by the

same fraction of $\Delta A/A$. As a result, the internal energy is increased by $K_a(\Delta A)^2/2A$. Thus the free energy of the peptide-lipid system (per lipid) can be written as $F_{PL} = \varepsilon_s(P - P_I)/L + \varepsilon_I P_I/L + K_a(\delta A)^2/2A_L$. ε_s and ε_I are, respectively, the binding energy per peptide in the planar bilayer region and the pore energy per peptide. Minimization of F_{PL} with respect to P_I leads to a constant $(P - P_I)$ for $P/L > P/L^*$, hence the plateau for the membrane thickness. [It also leads to a linear relation between P_I and $1/(P/L)$ that was confirmed by the measurement of P_I by oriented circular dichroism [10,17].]

The peptide-induced internal membrane tension is $\sigma = K_a(A_P/A_L)(P/L)$ before P/L exceeds P/L^*. Using the experimental value of K_a [12], we found the internal membrane tension at P/L^* (Fig. 2) in the range of 5–15 mN/m. These values are coincident with the membrane tensions for lysis of pure lipid vesicles measured by micropipette aspiration [22]. Membrane lysis most likely starts with a porelike structure. Thus the coincidence of these two tension values supports the idea that peptides induce pores by creating an internal membrane tension. In agreement with this interpretation is the observation that the lipid vesicle with bound peptides requires a smaller external tension to reach the point of lysis [21]. It is as if the tension for lysis is the same for pure lipid vesicles and for vesicles with bound peptides, only that for the latter the tension for lysis is the sum of the peptide-induced internal tension and the external tension. This is similar to the case of electroporation where the rupture tension is the sum of the electric-filed induced tension and the external tension [23].

Once the peptide density exceeds P/L^*, the peptide-induced tension becomes $\sigma = K_a(A_P/A_L)(P - P_I)/L$. This will explain why the peptide-induced pores are stable, as, for example, observable by neutron diffraction. P_I is the total number of peptides adsorbed at the rims of the pores, $P_I = N_p\Gamma_\ell 2\pi R$, where N_p is the total number of pores and Γ_ℓ the line density of the peptide at the rims. Then the energy of a pore is given by

$$E_R = 2\pi R\gamma - \pi R^2\sigma_0 + (4/3)\pi^2 R^3\sigma_0(N_p/P)\Gamma_\ell, \quad (1)$$

where $\sigma_0 = K_a(A_P/A_L)(P/L)$. One may regard Eq. (1) as a Landau free energy with the order parameter R and with the effect of decreasing temperature replaced by the effect of increasing P/L. Besides $R = 0$ (no pores), there is now a second energy-minimum solution at radius $R_0 = (c_2/3c_3) + \sqrt{(c_2/3c_3)^2 - (c_1/3c_3)}$ due to the R^3 term (the coefficients of E_R have been abbreviated as c_1, $-c_2$, and c_3). If the model were correct, the numbers should make sense. In a typical example, the antimicrobial peptide magainin in the 3:1 mixture of dimyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol was studied by neutron diffraction [18]. At $P/L = 1/20$, N_p/L was found to be $1/260$. Independently, γ and Γ_ℓ were measured for detergent Tween 20 in the pores of stretched vesicles [2]. In general, γ decreases with the detergent concentration and then reaches a plateau. In the plateau region where γ is constant, $\Gamma_\ell \sim 2\ \mathrm{nm}^{-1}$. Taking into account the difference in molecular weight (1228 for Tween 20 and 2465 for magainin), we have $\Gamma_\ell \sim 1\ \mathrm{nm}^{-1}$ for magainin. Thus $c_2/c_3 = 3(P/L)/[4\pi(N_p/L)\Gamma_\ell] = 3.1$ nm. As mentioned above, $\sigma_0 \sim 10$ mN/m. Without detergents or peptides, the line tension was measured to be $\gamma \sim 10$ pN [3]. This would give $c_1/c_2 = 2\gamma/\sigma_0 \sim 2$ nm, or $c_1/c_3 \sim 6.2\ \mathrm{nm}^2$. At this value of γ, there is no real solution for R_0; in other words, pores would close. However, like detergents, peptides preferentially bind to the rim of the pore [3]. As shown by Puech *et al.* [2], detergents reduce the line tension by a factor of 3 or more to a plateau value. This reduction of γ would make c_1/c_3 small enough to allow for a real solution of R_0. Thus the solution for R_0 is in the range of 1–2 times $c_2/3c_3$, or 1–2 nm. This is exactly the range of radius in which the peptide-induced pores have been found by neutron diffraction [18–20] or by leakage experiments [24]. In comparison, the transient pores in stretched vesicles are typically in the range of 1–10 μm.

Reducing the line tension γ by increasing P/L has another effect. Decreasing c_1 in E_R [Eq. (1)] is analogous to decreasing temperature in a Landau free energy. It makes the ordered state, in this case the state of pores, the lowest-energy state. Thus there are two equilibrium states for peptides bound to a lipid bilayer, a state of no pores and a state of multiple pores of the same size. The transition between the two as a function of P/L resembles a phase transition.

H. W. H. was supported by NIH Grants No. GM55203 and No. RR14812, and by the Robert A. Welch Foundation; F. Y. C. was supported by the National Science Council (Taiwan) through Contract No. NSC91-2112-M-008-22.

*Corresponding author: hwhuang@rice.edu

[1] F. Brochard-Wyart, P. G. de Gennes, and O. Sandre, Physica (Amsterdam) **278A**, 32 (2000).
[2] P. H. Puech, N. Borghi, E. Karatekin, and F. Brochard-Wyart, Phys. Rev. Lett. **90**, 128304 (2003).
[3] E. Karatekin, O. Sandre, H. Guitouni, N. Borghi, P.-H. Puech, and F. Brochard-Wyart, Biophys. J. **84**, 1734 (2003).
[4] J. D. Litster, Phys. Lett. A **35**, 193 (1975).
[5] C. Taupin, M. Dvolaitzky, and C. Sauterey, Biochemistry **14**, 4771 (1975).
[6] M. Zasloff, Nature (London) **415**, 389 (2002), and references therein.
[7] G. Baumann and P. Mueller, J. Supramol. Struct. **2**, 538 (1974).
[8] H. W. Huang, Biochemistry **39**, 8347 (2000).

[9] J. C. Weaver and Yu. A. Chizmadzhev, Bioelectrochem. Bioenerg. **41**, 135 (1996).
[10] F.-Y. Chen, M.-T. Lee, and H.W. Huang, Biophys. J. **84**, 3751 (2003).
[11] H.W. Huang, Biophys. J. **50**, 1061 (1986).
[12] W. Rawicz, K. C. Olbrich, T. McIntosh, D. Needham, and E. Evans, Biophys. J. **79**, 328 (2000).
[13] W. Helfrich, Z. Naturforsch. **28C**, 693 (1973).
[14] H.W. Huang, J. Phys. II (France) **5**, 1427 (1995).
[15] K. Matsuzaki, O. Murase, N. Fukii, and K. Miyajima, Biochemistry **34**, 6521 (1995).
[16] F.-Y. Chen, W. C. Hung, and H.W. Huang, Phys. Rev. Lett. **79**, 4026 (1997).
[17] F.-Y. Chen, M.-T. Lee, and H.W. Huang, Biophys. J. **82**, 908 (2002).
[18] S. J. Ludtke, K. He, W. T. Heller, T. A. Harroun, L. Yang, and H.W. Huang, Biochemistry **35**, 13723 (1996).
[19] K. He, S. J. Ludtke, D. L. Worcester, and H.W. Huang, Biophys. J. **70**, 2659 (1996).
[20] L. Yang, T. A. Harroun, T. M. Weiss, L. Ding, and H.W. Huang, Biophys. J. **81**, 1475 (2001).
[21] M. L. Longo, A. J. Waring, L. M. Gordon, and D. A. Hammer, Langmuir **14**, 2385 (1998).
[22] K. Olbrich, W. Rawicz, D. Needham, and E. Evans, Biophys. J. **79**, 321 (2000).
[23] H. Isambert, Phys. Rev. Lett. **80**, 3404 (1998); P. Sens and H. Isambert, Phys. Rev. Lett. **88**, 128102 (2002).
[24] K. Matsuzaki, O. Murase, N. Fujii, and K. Miyajima, Biochemistry **35**, 11 361 (1996).