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Review

Molecular mechanism of antimicrobial peptides: The origin of cooperativity

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Abstract

Based on very extensive studies on four peptides (alamethicin, melittin, magainin and protegrin), we propose a mechanism to explain the cooperativity exhibited by the activities of antimicrobial peptides, namely, a non-linear concentration dependence characterized by a threshold and a rapid rise to saturation as the concentration exceeds the threshold. We first review the structural basis of the mechanism. Experiments showed that peptide binding to lipid bilayers creates two distinct states depending on the bound-peptide to lipid ratio P/L. For P/L below a threshold P/L^*, all of the peptide molecules are in the S state that has the following characteristics: (1) there are no pores in the membrane, (2) the axes of helical peptides are oriented parallel to the plane of membrane, and (3) the peptide causes membrane thinning in proportion to P/L. As P/L increases above P/L^*, essentially all of the excessive peptide molecules occupy the I state that has the following characteristics: (1) transmembrane pores are detected in the membrane, (2) the axes of helical peptides are perpendicular to the plane of membrane, (3) the membrane thickness remains constant for $P/L \geq P/L^*$. The free energy based on these two states agrees with the data quantitatively. The free energy also explains why lipids of positive curvature (lysoPC) facilitate and lipids of negative curvature (PE) inhibit pore formation.

Keywords: Threshold peptide concentration; Cooperative concentration dependence; Membrane thinning effect; Neutron in-plane scattering; Oriented circular dichroism; Two-state model

Contents

The interactions of antimicrobial peptides (AMP) with cells can be very complicated. (One valuable reference for AMP is the proceedings of the Ciba Foundation Symposium held in 1994 [1] which, in addition to the presentations, recorded the discussions by the pioneers of the field on wide varieties of issues pertaining to AMP research that are usually excluded from journal articles, for example, the complexities of antimicrobial activity assays.) Because AMP are strongly cationic, they can potentially bind to many anionic groups present on the cell surface. Were it not for the following two empirical findings, it would be very difficult to discuss the mechanism of AMP activity: (1) the body of evidence overwhelmingly suggests that the target site of AMP is the lipid

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matrix of cytoplasmic membranes [1,2] and (2) the bioactivity of AMP correlates with the leakage from lipid vesicles induced by the same AMP [1–5]. For these AMP, it is then meaningful to study their interactions with pure lipid bilayers.

Both the bio- and leakage activities induced by AMP are characterized by cooperativity (often described as all-or-none), namely, a non-linear concentration dependence characterized by a threshold and a rapid rise to saturation as the concentration exceeds the threshold [3–6]. It is important to note that this includes both the bactericidal and hemolytic activities of AMP, only that in most cases, the lethal concentrations for hemolysis are two orders of magnitude higher than that for bactericide [3,4]. This difference is understandable because AMP being cationic are attracted to the negatively charged lipids on the outer leaflets of bacterial membranes, whereas such electrostatic effect is absent for mammalian membranes, whose outer leaflets are electrically neutral. Indeed a careful analysis by Wieprecht et al. [7] showed that if the bulk peptide concentrations are replaced by surface concentrations (i.e., excluding the electrostatic effect), similar binding constants and similar threshold concentrations were obtained for neutral and negatively charged membranes. In solution, the AMP thresholds for killing microbes are in the range of micromolar, whereas the thresholds for hemolysis are in the range of hundreds of micromolar [3,4]. It is their strongly cooperative activities that make the AMP effective antibiotics in the micromolar range without harming the host cells. Therefore, a proposed mechanism for the AMP activity must explain the origin of the cooperative concentration dependence.

The mechanism proposed below is based on very extensive studies on four peptides: alamethicin, melittin, magainin and protegrin. Although we will show only the data from our own laboratory, we will point out that our results are consistent with the data produced independently by other investigators using entirely different experimental methods and instruments.

The four peptides are among the most commonly studied due to their availability and relative simplicity. Alamethicin [8] and melittin [9] were discovered from the fungus *Trichoderma viride* and from the bee venom, respectively, in the late 1960s, and have been commercially available since 1970s. Initially, they were studied as the molecular models for voltage-gated channels (see review [10] for alamethicin, and [11] for melittin). The discoveries of AMP from animals in the 1980s, in particular magainins in the skin of *Xenopus laevis* [12], refocused the research on membrane-active peptides to their antimicrobial mechanisms [1]. Alamethicin, melittin and magainin are 20 to 26 amino acids long peptides; each forms a helical configuration when bound to lipid bilayers. The large number of studies on these peptides may have to do with the relative simplicity of their molecular structures. But it should be stressed that there is a great deal of similarities between the activity of magainins and that of cecropins from insects [3,4,12], although the latter are larger peptides and have more complex structures. This motivated us to compare helical peptides with non-helical peptides. Protegrin is an 18 amino acid peptide from the leukocytes of pigs [13]. Its configuration is a β-hairpin stabilized by two disulfide bonds. We have performed the same set of experiments on these four peptides and found that the results of all four peptides are consistent and similar to one another (references below). Thus we believe that the molecular mechanisms of cooperativity are essentially the same for these four peptides even though the details might differ.

1. Structural basis for the mechanism

1.1. Detection of pores

Do the peptides induce well-defined pores in membranes or disintegrate the membranes? To investigate this problem, we first bind peptides to lipid vesicles in various concentrations. We then evaporated most of the water, so the lipid vesicles with bound peptides flattened to become oriented multi-lamellae on a flat quartz surface. A simpler method for preparing such samples consists of (1) co-dissolve the lipid

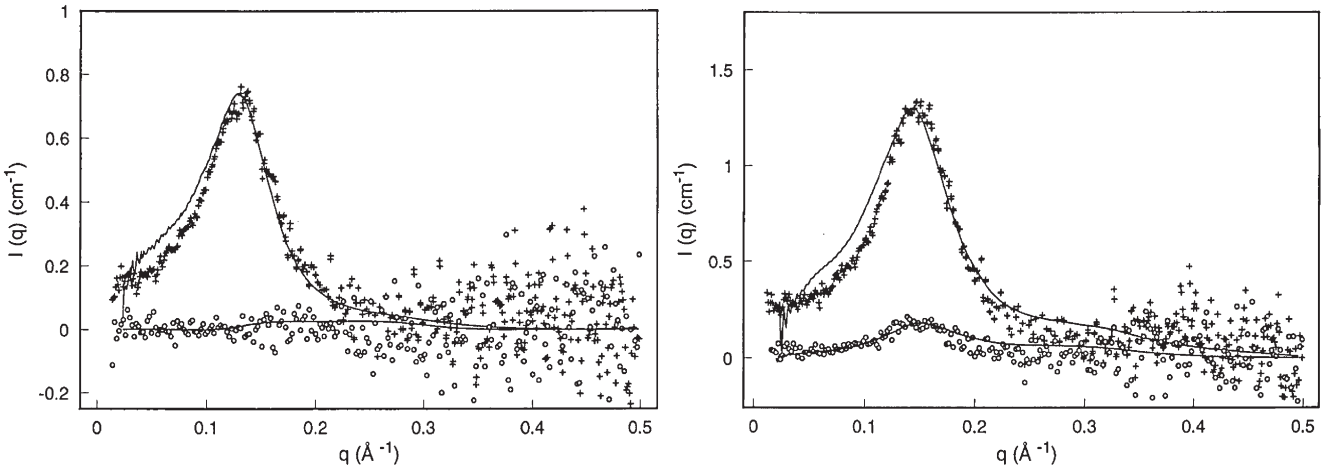

Fig. 1. Neutron in-plane scattering curve of alamethicin pores in DLPC bilayers at $P/L = 1/10$. (Left) The data (+) were obtained when hydrated with D_2O. After the sample was exposed to H_2O vapor for 48 h, the neutron scattering curve was indistinguishable from the background (○). The solid lines are the theoretical curves of 8-monomer barrel-stave pores in D_2O or H_2O. (Right) The sample condition was the same as (Left) except that the lipid was DLPC with fully deuterated chains, hydrated with H_2O (+) or D_2O (○). The solid lines are the theoretical curves of 8-monomer barrel-stave pores (from [18]).