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Voltage-dependent energetics of alamethicin monomers in the membrane

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Abstract

The implicit membrane model IMM1 is extended to include the effect of transmembrane potential and used to investigate the optimal membrane binding configurations and energies for alamethicin helices. In the absence of voltage, the lowest energy configuration is on the membrane surface with a tilt allowing the N terminus to be fully buried. Slightly higher in energy is an also tilted configuration with the N terminus deeper in the membrane and almost crossing the membrane. In 26 Å membranes and in the presence of 0.1 V voltage, the TM orientation becomes lower in energy. This is consistent with the assumption that voltage induces a transition from the interfacial to the inserted (TM) orientation. This effect of voltage is smaller in thicker membranes. The results are compared to previous experimental and theoretical studies and the findings are discussed in relation to the mechanism of channel formation by alamethicin.

Keywords: Transmembrane potential; Alamethicin; Voltage-dependent gating; Implicit membrane model

1. Introduction

Peptaibols are fungal peptides rich in α-aminoisobutyric acid (Aib) and include over 300 currently known sequences (see http://www.cryst.bbk.ac.uk/ peptaibol). The best studied of these is alamethicin (ALM), which forms weakly cation-specific ion channels in membranes and has served as a simple model for ion channels [1,2]. The structure of ALM in crystals is a bent α-helix [3]. Conductivity is voltage dependent and appears in bursts of different magnitude, which suggests that transient pores may be forming consisting of a variable number of monomers. Ion conductivity is largest when ALM is added to the +side of the membrane.

The most widely accepted mechanism for alamethicin action is the "barrel-stave" model, in which several ALM helices in an orientation parallel to the membrane normal cluster together forming a cylinder filled with water through which ions can flow. Biophysical studies with model membranes find either an interfacial [4] or a transmembrane [5] orientation depending on conditions [6]. Various propositions have been made for the voltage dependent step: partition into the bilayer, transition from an interfacial to a transmembrane orientation, conformational change, further immersion into the bilayer, flipping of helices from an antiparallel to a parallel orientation, or aggregation [2,7,8].

Quite a few molecular simulation studies have been reported, primarily by the Sansom group [9–18] but they have not reported relative free energies of different conformations. Continuum solvent studies predicted the transmembrane orientation to be slightly more favorable than the interfacial one [19]. Lately, research activity on ALM has somewhat abated, perhaps because existing techniques have reached their limits.

Despite the numerous studies on alamethicin and other peptaibols, a number of important questions still remain, for example: a) What is the voltage dependent step in ALM pore formation? b) Does the barrel-stave mechanism apply to the 16-residue peptaibols, which seem to be too short to span the bilayer? Some of them exhibit multiple level conductance states, like alamethicin, and some do not [1]. c) What is the role of the conserved Pro and the resulting kink in the middle of peptaibols? Analogs with the Pro replaced by Ala exhibited similar voltage gating but a smaller number of monomers per channel and shorter open channel lifetimes [8]. This work addresses the first of the above questions. We extend the IMM1 implicit membrane model to incorporate transmembrane potential using an analytical solution of the Poisson Boltzmann

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equation for planar membrane potential and apply it to the monomeric state of Alamethicin.

2. Methods

Over the past 3 years we have developed an effective energy function for proteins in lipid membranes [20] based on the EEF1 energy function for water-soluble proteins [21,22]. Effective energy (or potential of mean force, W) is the free energy of a given, fixed protein conformation and is equal to the intramolecular energy, obtained from a standard force field, plus the solvation free energy:

$$W = E + \Delta G^{\mathrm{slv}} \tag{1}$$

E is obtained from the extended atom (param19) CHARMM force field [23,24], where all atoms are represented explicitly except for the hydrogens that are bonded to nonpolar carbons. The theoretical foundation of the concept of effective energy and the distinction between effective energy and free energy are discussed in Ref. [25].

EEF1 aims to describe the effective energy of proteins in water. It differs from the standard CHARMM force field in two ways: a) an implicit solvation term is added that describes the interaction of each atom with the solvent, and b) the ionic sidechains are neutralized and a distance dependent dielectric constant ($\varepsilon = r$) is used for the electrostatic interactions. The implicit solvation term has the form:

$$\Delta G^{\mathrm{slv}} = \sum_i \Delta G_i^{\mathrm{slv}} = \sum_i \Delta G_i^{\mathrm{ref}} - \sum_i \sum_{j \neq i} f_i(r_{ij}) V_j \tag{2}$$

where $\Delta G_i^{\mathrm{slv}}$ is the solvation free energy of atom i and r_{ij} is the distance between i and j. Eq. (2) says that the solvation free energy of atom i is equal to that in a small model system where the atom is fully exposed to solvent ($\Delta G_i^{\mathrm{ref}}$) minus the solvation free energy it loses due to the presence of surrounding atoms. The solvation free energy density f is modeled as a Gaussian function

$$f_i(r) 4\pi r^2 = \alpha_i \exp(-x_i^2), \qquad x_i = \frac{r - R_i}{\lambda_i} \tag{3}$$

where R_i is the van der Waals radius of i, λ_i is a correlation length (3.5 Å for most atoms), and α_i is given by

$$\alpha_i = 2\Delta G_i^{\mathrm{free}} / \sqrt{\pi} \lambda_i \tag{4}$$

where $\Delta G_i^{\mathrm{free}}$ is the solvation free energy of the free (isolated) atom i; $\Delta G_i^{\mathrm{free}}$ is close but not identical to $\Delta G_i^{\mathrm{ref}}$ and is determined by requiring that the solvation free energy of deeply buried atoms be zero.

Calculations of the potential of mean force between ionizable sidechains in water [26] showed that EEF1 overestimates the attraction between unlike charged and hydrogen bonding groups. In addition, as was originally reported [21], certain interactions involving arginine were not correct. Thus we proposed a modified set of partial charges for the ionizable and polar sidechains designed to optimize the agreement with the calculated potentials of mean force. This updated version of the energy function is referred to as EEF1.1.

IMM1 (Implicit Membrane Model 1) is an extension of EEF1 to heterogeneous membrane–water systems. For molecules immersed in a membrane the values $\Delta G_i^{\mathrm{ref}}$ must correspond to a nonaqueous phase. Since data for the solvation free energy of molecules in the hydrocarbon core of membranes are not available (such data are available for partition at the membrane interface [27]), we used data for the distribution of amino acid sidechain analogues between cyclohexane and the gas phase [28]. The membrane is considered to be parallel to the xy plane with its center at $z = 0$. The solvation parameters of all atoms ($\Delta G_i^{\mathrm{ref}}$ and $\Delta G_i^{\mathrm{free}}$) now depend on the vertical direction, z, or $z' = |z|/(T/2)$, where T is the thickness of the nonpolar core of the membrane:

$$\Delta G_i^{\mathrm{ref}}(z') = f(z') \Delta G_i^{\mathrm{ref,\ wat}} + (1 - f(z')) \Delta G_i^{\mathrm{ref,\ chex}}. \tag{5}$$

The function $f(z')$ describes the transition from one phase to the other:

$$f(z') = \frac{z'^n}{1 + z'^n} \tag{6}$$

where n controls the steepness of the transition. The exponent $n = 10$ gives a region of 6 Å over which the environment goes from 90% nonpolar to 90% polar. This corresponds roughly to X-ray and neutron diffraction data for the structure of the lipid bilayers [29]. The midpoint of the transition ($f = 0.5$) corresponds to the hydrocarbon–polar headgroup interface. Hence, as in most other hydrophobic slab models, the headgroup region is assumed to have the same properties as aqueous solution.

To account for the strengthening of electrostatic interactions in the membrane in a way that is compatible with the distance dependent dielectric used in EEF1 we introduced a modified dielectric screening function

$$\varepsilon = r^{f_{ij}} \tag{7}$$

where f_{ij} depends on the position of the interacting atoms with respect to the membrane. Far from the membrane, f_{ij} is equal to 1 so that we recover the linear distance-dependent dielectric model. The model

$$f_{ij} = \sqrt{f_i f_j} \tag{8}$$

with f_i, f_j given by Eq. (6), which gives $\varepsilon = 1$ in the center of the membrane was found to strengthen electrostatic interactions too much. Therefore, we employed the empirical model

$$f_{ij} = a + (1 - a)\sqrt{f_i f_j} \tag{9}$$

with a an adjustable parameter. It was found that the value $a = 0.85$ gives reasonable results.

IMM1 was recently extended to account for the presence of net charge on the membrane surface (surface potential) [30]. The effect of surface potential was described using the Gouy–Chapman theory, which assumes that the charge is smeared on the membrane, solves analytically the 1-dimensional Poisson–Boltzmann equation, and yields the electrostatic potential as a