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Erratum

A thermodynamic approach to alamethicin pore formation

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ABSTRACT

The structure and energetics of alamethicin Rf30 monomer to nonamer in cylindrical pores of 5 to 11 Å radius are investigated using molecular dynamics simulations in an implicit membrane model that includes the free energy cost of acyl chain hydrophobic area exposure. Stable, low energy pores are obtained for certain combinations of radius and oligomeric number. The trimer and the tetramer formed 6 Å pores that appear closed while the larger oligomers formed open pores at their optimal radius. The hexamer in an 8 Å pore and the octamer in an 11 Å pore give the lowest effective energy per monomer. However, all oligomers beyond the pentamer have comparable energies, consistent with the observation of multiple conductance levels. The results are consistent with the widely accepted "barrel-stave" model. The N terminal portion of the molecule exhibits smaller tilt with respect to the membrane normal than the C terminal portion, resulting in a pore shape that is a hybrid between a funnel and an hourglass. Transmembrane voltage has little effect on the structure of the oligomers but enhances or decreases their stability depending on its orientation. Antiparallel bundles are lower in energy than the commonly accepted parallel ones and could be present under certain experimental conditions. Dry aggregates (without an aqueous pore) have lower average effective energy than the corresponding aggregates in a pore, suggesting that alamethicin pores may be excited states that are stabilized in part by voltage and in part by the ion flow itself.

1. Introduction

Alamethicin (ALM) is a 20-residue antimicrobial peptide produced by the soil fungus *Trichoderma viride* that is rich in α-amino isobutyric acid (Aib) and forms voltage-gated ion channels [1,2]. The crystal structure of ALM showed an α-helix from the N-terminus to Pro 14 and a 3_{10} helix from Pro 14 to the C-terminus [3]. The fact that ALM-induced ion conductivity appears at discrete levels [4] has been attributed to formation of transient "barrel-stave" pores consisting of a variable number of monomers around a central aqueous pore [5,6]. Various propositions have been made for the voltage dependent step: partition into the bilayer, transition from an interfacial to a transmembrane (TM) orientation, conformational change, further immersion into the bilayer, flipping of helices from an antiparallel to a parallel orientation, or aggregation [2,7,8].

A variety of experimental techniques have been employed to understand the mechanism of ion channel formation by ALM. Its ion conductance properties have been investigated under different conditions [9–11], including covalent tethering [12]. Some studies with model membranes suggested an interfacial orientation [13–15], while others found a TM orientation [16,17], a highly tilted orientation [18], or a distribution of orientations [19]. Other studies detected both orientations depending on peptide concentration and hydration [20–22]. Conflicting findings have also been reported on the aggregation state of ALM in membranes in the absence of voltage, with some studies finding predominantly monomers [23–25], while others detected oligomers [26–30], with aggregation diminishing at higher temperatures [31]. Low-resolution information on the structure of the ALM pore has been obtained by neutron scattering. It was found that in DLPC the pores are made of 8–9 helical peptides arranged in parallel around an 18 Å diameter water-filled pore [32]. Somewhat larger pores were obtained in DPhPC. Indirect information on the pore size has also been obtained by studying the effect of polymers on the observed conductance [33]. The barrel stave model is widely accepted, but not universally [10,14].

ALM has also been the subject of numerous theoretical studies. Models of the channel have been constructed with restrained molecular dynamics (MD) in an implicit bilayer [34] or in vacuum with a few explicit water molecules [35]. Explicit solvent MD simulations have also been performed on monomers in water and/or methanol [36,37] and inserted [37,38] or adsorbed [39,40] on lipid bilayers or octane slabs. Simulations of ALM oligomers in lipid bilayers have also been performed [41–43]. The authors suggested that the tetramer does not conduct ions and that the lowest conductance level likely corresponds to a pentamer. Most stable in the simulations was found to be the hexamer. A more recent coarse-grained and atomistic MD study found extensive aggregation of ALM in a lipid bilayer [44]. The peptides exhibited occasional transitions between the membrane spanning and the surface

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bound configurations. Continuum electrostatics analysis showed the TM orientation to be more stable than the interfacial orientation [45] and that many channel structures obtained by MD are thermodynamically unstable in the membrane [46].

In previous work from our group, MD simulations in implicit membrane produced two possible orientations of similar energies: a tilted orientation at the interface with the N terminus partially inserted and a more fully inserted, TM orientation with the N terminus almost crossing the membrane [47]. The transfer energy from water to the membrane was large enough to ensure that all ALM partitioned to the membrane at reasonable concentrations. These results were in agreement with a variety of experimental studies suggesting that ALM penetrated the hydrophobic core of the bilayer even in the absence of voltage [1]. Also, that the TM orientation did not completely cross the membrane was in agreement with a spin-labeling EPR study [48]. Voltage did not produce significant change in these structures but shifted the equilibrium towards the TM orientation by 0.8–0.9 kcal/mol [47]. More recently we found that inclusion of the membrane dipole potential [49] or lateral pressure effects [50] makes the interfacial configuration more parallel to the membrane.

Although ALM has been studied extensively, the pore structure in the membrane is still under debate. All atom MD studies have probed the kinetic stability of model pore structures, but have not yet provided information on their thermodynamic stability due to the large computational expense. Here, we attempt to do just that by introducing new methodology based on implicit membrane modeling. We construct models of different oligomeric numbers around pores of different sizes and compute the average effective energy per monomer. The implicit model also allows us to easily assess the effect of external voltage and evaluate proposed mechanisms of voltage dependence. Further, comparison with the energies of dry aggregates allows us to investigate the nature of the closed state and the energetics of the opening transition.

2. Methods

2.1. Implicit membrane model

We performed MD simulations with IMM1, an effective energy function for proteins in lipid membranes [51], which is an extension of EEF1 for water-soluble proteins [52]. Effective energy (W) is the free energy of a given, fixed protein conformation and is obtained as the sum of the intramolecular energy (E) and the solvation free energy (ΔG^{slv}). EEF1 uses the extended atom CHARMM force field (param19) [53] with neutralized ionic side chains and a linear distance dependent dielectric constant ($\varepsilon = r$) for the electrostatic interactions. IMM1 extends EEF1 to heterogeneous membrane–water systems by allowing the solvation parameters to vary between values corresponding to aqueous solution and values corresponding to cyclohexane. The membrane is considered to be parallel to the xy plane with its center at $z = 0$. The solvation parameters of all atoms (ΔG_i^{ref}) depend on the vertical position, $z' = |z| / (T/2)$, where T is the thickness of the nonpolar core of the membrane. To account for the strengthening of electrostatic interactions in the membrane, a modified dielectric screening function is used

$$\varepsilon = r^{f_{ij}} \quad f_{ij} = a + (1-a)\sqrt{f_i f_j} \tag{1}$$

where f_i and f_j are given as

$$f\left(z'\right) = \frac{z'^n}{1+z'^n}. \tag{2}$$

The switching function f describes the transition from one phase to the other and n controls the steepness of the transition. The exponent $n = 10$ gives a region of 6 Å over which the environment transitions from 90% nonpolar to 90% polar. The value 0.85 for the adjustable parameter a was found to give membrane binding energies in accord with experiment. Modeling of proteins with an aqueous pore was made possible by making the switching function dependent on the distance from the z axis [54,55]:

$$F\left(z',r'\right) = f\left(z'\right) + h\left(r'\right) - f\left(z'\right) + h\left(r'\right) \quad , h(r) = 1 - \frac{r'^n}{1+r'^n}, \quad r' = r/R, \quad r = \sqrt{x^2+y^2} \tag{3}$$

where r is the distance of any atom from the center of the pore and R the radius of the pore. The resulting energy function was referred to as IMM1-pore and was shown to discriminate the correct fold of TM beta barrels [54].

2.2. Extension to the all-atom CHARMM 36 force field

The EEF1 and IMM1 functions are based on the "united atom" CHARMM 19 force field in which the nonpolar hydrogen atoms are not explicitly represented. While this approximation is reasonable, it may affect the packing energies [56]. Indeed, interactions between TM helices sometimes appear too strong with this energy function. In addition, it does not allow one to take advantage of the progress in parameterization that took place over the last 15 years. For these reasons, IMM1 was adapted to the most recent, all-atom force field, referred to as CHARMM 36 [57]. This was done by transferring the solvation parameters from the atom types of CHARMM 19 to the corresponding atom types in CHARMM 36 and modifying the partial charges of the ionizable residues in CHARMM 36 to match those in IMM1.

One more change was necessary. CHARMM 19 scales the 1–4 interactions (the interactions between atoms separated by three bonds) by 0.4, while the all-atom CHARMM force fields do not scale them at all. This affects significantly the transfer energies from water to the membrane because IMM1 uses a position-dependent dielectric constant for all electrostatic interactions (Eq. (1)). Without the scaling of 1–4 interactions, IMM1 gives very little electrostatic stabilization in the membrane. Thus, the code was modified so that 1–4 interactions are excluded from the scaling in Eq. (1) and the value of the parameter a was adjusted to 0.91 to obtain roughly the same electrostatic stabilization in the membrane as with the original IMM1. This version is referred to as IMM1-p36.

2.3. Free energy of hydrophobic exposure

In its standard form, IMM1-pore produced distorted ALM oligomeric structures in cylindrical pores with monomers highly tilted. We hypothesized that this is due to the neglect of the free energy cost of hydrophobic exposure of lipids when the pore is not completely lined with peptide. To include this free energy cost, the effective energy (W) was modified as follows:

$$W = E + \Delta G^{slv} + E^{pore} \tag{4}$$

where E^{pore} is the residual pore energy and it is expressed as

$$\frac{E^{pore}}{\gamma} = 2\pi RT - \sum_i \left(1 - f\left(z_i'\right)\right) \exp\left(-\frac{(r_i - R)^2}{\beta}\right) \pi r_i^{vdw^2} \tag{5}$$

where γ is the hydrocarbon–water interfacial tension, for which we chose the value 50.05 mN/m [58], and R is the radius of the pore. The first term on the right-hand side, multiplied by γ, corresponds to the classical pore formation energy for a membrane with thickness T. This is the energy of the "naked" pore (no peptides present). The second term in Eq. (5) represents the lowering of the free energy due to