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Competing interactions for antimicrobial selectivity based on charge complementarity

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ABSTRACT

Antimicrobial peptides (AMPs) are an evolutionary conserved component of the innate immune system and possible templates for the development of new antibiotics. An important property of antimicrobial peptides is their ability to discriminate bacterial from eucaryotic cells which is attributed to the difference in lipid composition of the outer leaflet of the plasma membrane between the two types of cells. Whereas eucaryotic cells usually expose zwitterionic lipids, procaryotic cells expose also anionic lipids which bind the cationic antimicrobial peptides electrostatically. An example is the antimicrobial peptide NK-2 which is highly cationic and favors binding to anionic membranes. In the present study, the difference in binding affinity of NK-2 for palmitoyl-oleoyl-phosphatidyl-glycerol (POPG) and palmitoyl-oleoyl-phosphatidyl-choline (POPC) is studied using molecular dynamics simulations in conjunction with a coarse grained model and thermodynamic integration, by computing the change in free energy and its components upon the transfer of NK-2 from POPC to POPG. The transfer is indeed found to be highly favorable. Interestingly, the favorable contribution from the electrostatic interaction between the peptide and the anionic lipids is overcompensated by an unfavorable contribution from the change in lipid–cation interactions due to the release of counterions from the lipids. The increase in entropy due to the release of the cations is compensated by other entropic components. The largest favorable contribution arises from the solvation of the counterions. Overall the interaction between NK-2 and POPG is not determined by a single driving force but a subtle balance of competing interactions.

1. Introduction

AMPs are part of the innate immune system among various types of species, including mammals, amphibians, fish, insects, birds and plants [1]. Their toxic activity focuses on Gram-negative as well as Gram-positive bacteria, fungi, viruses, parasites, and also mammalian cells. Besides their natural relevance, AMPs have become of interest as templates for the development of new antibacterial drugs, as bacteria get increasingly resistant against classical antibiotics. Hence, understanding the function of AMPs in detail is of great biophysical and medical importance.

The traditional characteristics of AMPs include their small size, typically <10 kDa, amphiphilic properties and a positive charge [2,3]. A lot of studies focus on the largest group of AMPs which induce pores in the cytoplasmic membrane of bacteria leading to their lysis and cell death. This group of AMPs can discriminate between eucaryotic and procaryotic cells due to their different lipid composition. A procaryotic cytoplasmic membrane typically contains the anionic lipid cardiolipin and phospholipids with either an anionic PG or a zwitterionic PE headgroup [4]. The outer leaflet of a eucaryotic cell membrane typically contains sphingomyelin or lipids with the zwitterionic head group PC [5]. In vitro experiments [6,7] using model membranes show that AMPs bind more strongly to PE and even more strongly to anionic lipids but show negligible interactions with PC lipids. Antimicrobial selectivity hence seems to arise from the abundance of PE or anionic lipids in procaryotic and PC lipids in eucaryotic membranes, in particular, the strong interaction of the highly cationic AMPs with the anionic lipids based on charge complementarity. The view that mainly the anionic lipids exposed by bacteria lead to strong interactions with antimicrobial peptides is also supported by the finding that bacteria with particularly high concentrations of negatively charged lipids in the outer leaflet are especially susceptible to AMPs [8].

The antimicrobial protein peptide NK-lysin (PDB ID: 1NKL) is expressed in porcine natural killer ("NK") cells. It consists of 78 residues (~9 kDa) and forms five α-helical segments. A high antimicrobial activity and selectivity has been found for a peptide derived from the

Abbreviations: POPG, palmitoyl-oleoyl-phosphatidyl-glycerol; POPC, palmitoyl-oleoyl-phosphatidyl-choline; MD, molecular dynamics; AMPs, antimicrobial peptides; PG, phosphatidyl-glycerol; PE, phosphatidyl-ethanolamine; PC, phosphatidyl-choline; DPPC, diplamitoyl-PC; DPPG, diplamitoyl-PG; DPPE, diplamitoyl-PE; LJ, Lennard–Jones; LINCS, linear constraint solver for molecular simulations; NT, N-terminus; NC3, choline; PO_4, phosphate

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sequence of the third and fourth α-helix consisting of residues 39 to 65 [9]. Three point mutations (L44V, S51T, and W58K) of this segment leading to the amino acid sequence KILRGVCKKIMRTFLRRISKDILTGKK and amidation of the C-terminus yield a peptide with a net charge of +10; this peptide is denoted as NK-2 [9]. Experiments display activity of NK-2 against Gram-positive and Gram-negative bacteria but not against human red blood or human skin cells [9]. NK-2 shows negligible interactions with DPPC or POPC but significantly interacts with DPPG or POPG model membranes in experiment [10–12]. In particular, Willumeit et al. studied the zeta potentials of DPPC, DPPE, and DPPG vesicles in the presence of NK-2 as a function of the peptide:lipid ratio [11]. Whereas increasing the peptide:lipid ratio did not affect the zeta potentials of DPPC vesicles, it rendered the zeta potentials of DPPE and DPPG more positive. The largest effect was observed for DPPG vesicles, whose zeta potentials increased from −60 mV in the absence of NK-2 to +30 mV in the presence of NK-2 at a peptide:lipid ratio of 1:2. The charge reversal indicates that the strong attraction of NK-2 to DPPG is not only driven by electrostatics alone. As the peptide is amphiphilic, hydrophobic interactions could also play a role. However, such interactions could also form with DPPC, such that the full mechanism underlying antimicrobial selectivity cannot be deduced from these experiments.

A powerful tool to understand the binding of AMPs to model membranes on a microscopic level is provided by MD simulations. Sayyed-Ahmad et al. [13] found that relative affinities of antimicrobial peptides for micelles from zwitterionic single hydrocarbon chain lipids mimicking the interfacial electrostatics of the outer leaflet of eucaryotic plasma membranes correlated with the rankings for the peptides' hemolytic toxicities observed experimentally. On the other hand, relative affinities of antimicrobial peptides for micelles from anionic single hydrocarbon chain lipids correlated with the experimentally measured ranking for the antimicrobial activities of the peptides. In these studies, the affinities were computed by performing MD simulations of the systems in water and analyzing the trajectories using molecular mechanics gas phase energies and solvation free energies from continuum models.

Absolute binding affinities of a peptide for a membrane or an interface may be obtained from simulations where the peptide is restrained at different distances from the membrane [14–18]. Horinek et al. used this technique to study the interaction of a mildly hydrophobic peptide with a solid surface [14]. Davis and Berkowitz employed this method to investigate the affinity of the Alzheimer disease-related amyloid $\beta(1–42)$ peptide for membranes with different lipid compositions [15]. Gray et al. studied affinities of the antimicrobial peptide lactoferricin for POPC and POPG bilayers and found the peptide to bind more strongly to PG [16,17]. The strong binding to POPG was partly attributed to the gain in entropy due to the release of counterions [16,17]. A similar method was applied to study the binding of the cationic β-hairpin antimicrobial peptide protegrin-1 to bilayers composed of a mixture of POPG and POPE; here, the affinity of the peptide for the bilayer was attributed exclusively to the entropy gain due to counterion release [18].

Pimthon et al. conducted MD simulations of NK-2 attached to a DPPE or a DPPG bilayer at ambient conditions where both lipids form a gel phase [19]. It was found that NK-2 binds to the interface between the lipid head groups and the water with its hydrophobic side pointing toward the interior of the membrane; NK-2 was observed to penetrate somewhat more deeply into the head group region of DPPG than into that of DPPE, such that the peptide slightly interacts with the hydrocarbon tails of DPPG but not with those of DPPE, suggesting that hydrophobic interactions may support the stronger binding of NK-2 to DPPG compared to a zwitterionic bilayer. However, Pimthon's study referred to bilayers in the gel state whereas the biologically relevant phase is the fluid phase.

Here we have used MD simulations to compare the binding of NK-2 to POPC and to the anionic lipid POPG, both lipids being in the fluid phase at ambient conditions. Calculations of absolute binding affinities may suffer from a sampling problem for the solvated state of the peptide, as circular dichroism studies indicate that NK-2 in aqueous solution is disordered such that its configurational space might be vast [12]. Instead, membrane-bound NK-2 is α-helical [12]. A coil–helix transition upon adsorption at a membrane is observed for many antimicrobial peptides. Hence, we concentrate on the bound state and compare the affinity of NK-2 for POPC and POPG by computing the free energy change upon the transfer of NK-2 from POPC to POPG using thermodynamic integration. In order to be able to sample sufficiently long timescales, a coarse grained model is employed. The procedure is similar to that chosen previously to compare POPC with POPE [20]. We find that the free energy of transfer is strongly negative, indicating that NK-2 binds much more strongly to PG than to PC, in agreement with experimental results [10–12]. As found previously for the free energy change upon the transfer of NK-2 from PC to *PE* [20], the transfer free energy is found to be governed by a complex interplay of competing interactions. In particular, the favorable electrostatic interactions due to the interaction of the cationic peptide with the anionic POPG lipids are overcompensated by an unfavorable electrostatic contribution due to the release of counterions from the peptide and the lipids. However, the counterion release leads to a strong favorable contribution from ion hydration.

2. Methods

2.1. Setup

The systems studied here included a monocomponent phospholipid bilayer of 128 phospholipids and approximately 2700 water molecules simulated under periodic boundary conditions. The phospholipid was either POPC or POPG. Both peptide-free bilayers and bilayers attached to a single NK-2 molecule were considered. The charge of the cationic peptide was neutralized with ten counteranions and the charge of the 128 POPG lipids with 128 cations. A typical configuration of the POPC and the POPG bilayer with NK-2 and the counterions is shown in Fig. 1. The systems were described using the MARTINI coarse grained model [21,22] which is based on a four-to-one mapping of heavy atoms to coarse grained beads. In this model, known or assumed secondary structure elements of peptides are stabilized by appropriate constraints. Circular dichroism spectra and molecular dynamics simulations suggest that NK-2 is α-helical when attached to phospholipid bilayers [19,12]. Hence, the helix–kink–helix structure of the sequence within the parent protein NK-lysin was maintained via dihedral potentials on the backbone stabilizing α-helical conformations except for the residues Thr-13 and Phe-14 representing the kink.

A single POPC lipid molecule in coarse grained representation is displayed in Fig. 2, where the *Sn*-1 chain is the saturated tail and consists of four particles. The *Sn*-2 chain is the unsaturated tail with the particle in the third position exhibiting a double bond character.

The simulation protocol was chosen according to standard settings for the MARTINI model. In this model, the polarity of atomic groups is parameterized by effective Lennard–Jones potentials of the form

$$V_{LJ}(r) = \epsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r}\right)^{12} - \left(\frac{\sigma_{ij}}{r}\right)^{6}\right]. \quad (1)$$

Here, σ_{ij} denotes the closest distance of approach between two particles and ϵ_{ij} the strength of their interaction. The same effective size, $\sigma \equiv 0.47$ nm, is taken for each interaction pair except for the interactions between charged particles and (i) the lipid tails or (ii) leucine, isoleucine, or valine side chains for which $\sigma \equiv 0.62$ nm. The choline particle in POPC and the glycerol particle in POPG have the same effective size of 0.62 nm but differ in their interaction strength. The interaction parameter ε is larger for glycerol than for choline to