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The cooperative behaviour of antimicrobial peptides in model membranes

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ABSTRACT

A systematic analysis of the hypothesis of the antimicrobial peptides' (AMPs) cooperative action is performed by means of full atomistic molecular dynamics simulations accompanied by circular dichroism experiments. Several AMPs from the aurein family (2.5,2.6, 3.1), have a similar sequence in the first ten amino acids, are investigated in different environments including aqueous solution, trifluoroethanol (TFE), palmitoyloleoylphosphatidylethanolamine (POPE), and palmitoyloleoylphosphatidylglycerol (POPG) lipid bilayers. It is found that the cooperative effect is stronger in aqueous solution and weaker in TFE. Moreover, in the presence of membranes, the cooperative effect plays an important role in the peptide/lipid bilayer interaction. The action of AMPs is a competition of the hydrophobic interactions between the side chains of the peptides and the hydrophobic region of lipid molecules, as well as the intra peptide interaction. The aureins 2.5-COOH and 2.6-COOH form a hydrophobic aggregate to minimize the interaction between the hydrophobic group and the water. Once that the peptides reach the water/lipid interface the hydrophobic aggregate becomes smaller and the peptides start to penetrate into the membrane. In contrast, aurein 3.1-COOH forms only a transient aggregate which disintegrates once the peptides reached the membrane, and it shows no cooperativity in membrane penetration.

1. Introduction

Modern increase in antibiotic-resistance bacterial infections urges the development of new and non-conventional therapeutic agents with novel mechanisms of antimicrobial action [1,2]. Bioactive peptides, which are usually short molecules up to 50 amino acids, can be found in diverse range of organisms including plants, mammals, amphibians and insects. They are potential candidates to fulfil this role and some of the peptide antibiotics are currently in clinical trials [3–5].

How peptides with antimicrobial actions kill the bacteria is subject to continuous research [5–8]. Three different models for peptide-membrane interaction are commonly used: barrel-stave, toroidal and carpet models[9–11]. It was suggested that collective behaviour of peptides can play a role in the bacterial membrane destruction [12–19]. For instance, using ^{31}P oriented solid-state NMR experiments it was found that at high peptide concentration alamethicin adopts a transmembrane conformation while the novicidin forms a toroidal pore in the membrane [16]. Using solid-state ^{19}F NMR it was shown that at low concentration the amphiphilic $[KIGAKI]_3$ peptide binds to membrane as flexible β-strand, without forming any intra or intermolecular H-bonds [13]. At higher concentrations $[KIGAKI]_3$ self-assembles into immobilized β-sheets which lie flat on the membrane surface as amyloid-like fibrils. Combining fluorescence assay, SEM, and AFM characterisation Chen et al. [15] suggested a detergent-like mechanism of antimicrobial action where A_9K peptide self-assemble into the rod-like micelles, which pierce through the membrane leading to its lysis. In a recent experimental study a novel mechanism of peptide-induced cell lysis was proposed which is due to the peptide self-assembly into exosome-like aggregates [17]. Such self-assembly requires a strong collective behaviour of several antimicrobial peptides (AMPs).

Molecular dynamics (MD) simulations have been applied to understand the conformation and mechanism of AMPs, as well as related viral and cell-penetrating peptides [20,21]. MD simulation studies on the timescale of tens to hundreds of nanoseconds have successfully helped to model or refine the conformation of AMPs and their aggregation in the presence of membrane-mimicking solvent mixtures, detergent micelles, and lipid bilayers [22–32]. Several studies employed coarse-grained MD (CG-MD) to investigate the behaviour of peptide/

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complexes into lipid membrane [33–35] (see also an extensive review on CG-MD by Shinoda and collaborators) [36]. Using coarse-grained MD Sansom and co-workers found that the AMP maculatin 1.1. forms membrane-inserted aggregates, which allow for a water permeation through a fluctuating channel [34,37]. Using MD simulation Ref. [22] has shown that the peptide CM15 has a strong tendency to form α-helices inside in a ratio of 1:2 of palmitoyloleoylphosphatidylcholine (POPC) and palmitoyloleoylphosphatidylglycerol (POPG) membranes. Using MD Chen and Mark found that short peptides, aurein 1.2 and citropin 1.1, disrupt the membrane via a detergent-like mechanism inducing high local curvature while longer peptides such as maculatin 1.1 and the caerin 1.1 induce longer range curvature stabilizing the membrane pores [38]. Pourmousa et al. studied trasportan peptide in dipalmitoylphosphatidylcholine (DPPC) lipid bilayer finding that the lysine residue facilitates the process of diffusion of the peptide inside the membrane [27]. Investigating the behaviour of melittin in DPPC lipid bilayer Sengupta et al. found the formation of disordered toroidal pores at the high concentration of the peptide [19]. It has been shown that the charged residues of melittin play a crucial role in the pore formation in DPPC [19] and POPC [39]. Using the full atomistic MD in our previous work we found that the probability of penetration of AMP peptide aurein 2.3 inside the membrane is larger for higher AMP concentration [40]. However, a systematic study of the peptides' behaviour at higher concentrations with a full atomistic resolution in the presence of different environments is still lacking [7,41]. In this study we report combined MD and experimental results on single and multiple AMPs from aurein family. We have chosen a set of peptides with a broad range of antimicrobial activities (aurein 2.5-COOH, 2.6-COOH and 3.1-COOH). In order to investigate the influence of amino acid sequence on the cooperativity behaviour the choice was limited to the peptides, which have most of the sequences in common (starting ten amino acids). Moreover, the choice of aurein 3.1 was motivated by the presence of a polar histidine HIS residue instead of a hydrophobic amino acid (alanine or valine). The presence of histidine can change the peptide-membrane interaction and enhance or inhibit the peptide–peptide interaction. It was found that HIS-rich AMPs have a broad range of antimicrobial activity [42]. Therefore, their detailed investigation can help in designing new antimicrobial agents. In the present work the peptides were interacting with palmitoyloleoylphosphatidylethanolamine (POPE), and (POPG) lipid bilayers. The two membranes have different chemical properties: POPE is a zwitterionic lipid bilayer while POPG is an anionic lipid bilayer. POPE and POPG were chosen since they are the main components of Gram-positive bacteria, such as *Bacillus cereus*[43], some strains of which can cause severe foodborne diseases.

2. Materials and methods

2.1. Materials

Phospholipids 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) were obtained from AVANTI polar lipid and used without further modification. The peptide analogues of aurein peptides: aurein 2.5-COOH (GLFDIVKKVVGAFGSL-COOH), aurein 2.6-COOH (GLFDIAKKVIGVIGSL-COOH) and aurein 3.1-COOH (GLFDIVKKIAGHIAGSI-COOH) were synthesized by SevernBiotech by solid state synthesis and purified by HPLC to purity greater than 95%. 2,2,2-Trifluoroethanol (TFE) and all other solvents and reagents were supplied by Fisher Scientific UK.

2.2. Circular dichroism measurements

Circular dichroism (CD) was recorded on a J-815 spectropolarimeter (JASCO, UK) equipped with a peltier temperature control unit using a 10 mm path-length cell over a wavelength range of 260 to 180 nm at a scan speed of 50 nm/min, 1 nm band width, and data pitch 0.5 nm. Far-UV CD spectra were collated for each peptide (0.01 mg/ml) in H_2O, PBS buffer (pH 7.4) and 100% TFE. CD experiments were also performed at a peptide:lipid ratio 1:100. To obtain small lamellar vesicles (SUVs), a predetermined amount of dried (5 mg/ml) POPG and POPE were dissolved in chloroform, evaporated under a stream of nitrogen, placed under vacuum overnight. The lipid film was then rehydrated using 1 × phosphate-buffered saline (PBS, pH 7.5) and sonicated 1 h or until the solution was no longer turbid. SUVs were then extruded 11 times through a 0.1 μm polycarbonate filter using an Avanti polar lipid mini-extruder apparatus. All CD experiments were obtained by acquiring 10 scans on a J-815 spectropolarimeter (Jasco, UK) and samples maintained at 30 °C. For all spectra acquired, the baseline acquired in the absence of peptide was subtracted. The percentage α-helical content was then estimated using CDSSTR algorithm (protein reference set 3) on the DichroWeb server [44–46]. These experiments were repeated four times and the percentage helicity was averaged.

2.3. Simulations

The mechanism of interaction between each aurein analogue and either 0.1 mol/l aqueous solution, TFE, POPE, and POPG was examined using molecular dynamics (MD). The aurein peptide analogues were each assembled as canonical α-helix using AMBER tools 1.4. Simulations and the analysis have been performed using GROMACS [47,48]. The simple point-charge (SPC) water model has been used [49]. The GROMOS 53a6 force fields for POPE and POPG was employed [50,51]. All structures have been equilibrated at room temperature in water in the following sequence: minimization, NVT and NPT simulation. In all cases the peptides have been positioned at 3 nm from the top leaflet of the lipid bilayer with his axis perpendicular to the interface of lipid bilayer and water [40]. The counter ions Na^+ and Cl^-have been added to neutralize the systems. All structures have been equilibrated at 303 K in the sequence minimisation: NVT and NPT simulations. A 200 ns equilibration at 303 K has been performed. An equilibration run of 2 ns has been carried out for the peptide–lipid bilayer system with the position of the peptide restrained using harmonic restraints with a force constant of 1.0 kJ^{-1} nm^{-2} per atom[38,51,52]. The cut off for both van der Waals and Coulombic interactions is 1.2 nm. Berendsen temperature coupling is used at 303 K while the water and the bilayer were coupled separately with coupling time of 0.1 ps for single groups. A semi-isotropic Berendsen barostat is used with coupling time of 2.0 ps [50,51]. The main molecular dynamic simulations (no restraints) have been performed at constant temperature, pressure and number of molecules. In order to calculate the angle between the lipid bilayer and the peptide the post-processing tool with GROMACS is used. The trajectories have been generated by extracting the coordinates every 20,000 steps. Bond lengths have been constrained using the LINCS algorithm [53]. The MD simulations have been performed in the NPT ensemble using periodic boundary conditions. The components for each system are shown in Table 1.

3. Results

3.1. Secondary structure of aureins in solutions and in presence of lipid bilayer

3.1.1. Experiments

Secondary structure analysis was performed using CD spectral data. Fig. 1A shows the solution structure of aurein 2.5-COOH, aurein 2.6-COOH and aurein 3.1-COOH. Far-UV CD spectra of the three peptides in PBS buffer and in water environment at neutral pH showed an unordered structure. However, in the presence of TFE, CD spectra (Fig. 1B) show two minima at 220 nm and 207 nm and a maximum at 195 nm for each of the peptides, which is characteristic of α-helical structure. The estimated helical content is 28% for aurein 2.5-COOH, 75% for aurein 26-COOH and 63% for aurein 3.1-COOH. The presence of POPE