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Bacteriocin AS-48 binding to model membranes and pore formation as revealed by coarse-grained simulations

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ABSTRACT

Bacteriocin AS-48 is a membrane-interacting peptide that acts as a broad-spectrum antimicrobial against Gram-positive and Gram-negative bacteria. Prior Nuclear Magnetic Resonance experiments and the high resolution crystal structure of AS-48 have suggested a mechanism for the molecular activity of AS-48 whereby the peptide undergoes transition from a water-soluble to a membrane-bound state upon membrane binding. To help interpret experimental results, we here simulate the molecular dynamics of this binding mechanism at the coarse-grained level. By simulating the self-assembly of the peptide, we predict induction by the bacteriocin of different pore types consistent with a "leaky slit" model.

1. Introduction

Antimicrobial peptides (AMP) have a number of interesting properties that make them promising candidates for the treatment of infectious diseases and food preservation. Their mechanisms of action have been addressed in several experimental and simulation studies [1–21]. These studies have revealed, for example, that by having several mechanisms of enzyme inhibition, some AMPs are able to avoid the development of microbial resistance. In recent reviews of the structure and properties of AMPs [4,14], cyclic peptides in particular emerged as a special class of very stable active compounds [13].

In the late 1980s, AS-48, a cyclic 70-residue peptide showing remarkable stability and bactericidal activity, was isolated from bacteria by Galvez, Valdivia, Maqueda and coworkers [22–24]. Ten years later, NMR and X-ray crystallography revealed the dimeric structure of this bacteriocin [25–27]. The monomer (aka protomer) is composed of five alpha-helices enclosing a hydrophobic core in a compact globular structure (see Fig. 1). It is accepted that charged residues are mainly located in the α_4 and α_5 helices separate from the hydrophobic regions. Four negatively charged GLU residues form a patch that segregates the hydrophobic zone from an area of positively charged residues (blue regions in α_4 and α_5).

In aqueous solution, AS-48 peptides appear as dimeric form I or II (PDB ID: 1O83 and 1O84, respectively) depending on the chemical environment. Protomers arrange themselves in such a way that hydrophobic residues are buried in the dimer peptide core while charged residues interact with the surrounding water environment (see Fig. 2.a). When a detergent is added, the dimer adopts a contiguous organisation such that the hydrophobic moiety of each protomer remains in the hydrophobic phase while charged residues occupy the water phase (see Fig. 2.b).

According to this behaviour, it has been hypothesized [27] that, in solution, AS-48 adopts the configuration of dimeric form I and switches to dimeric form II upon binding to the bacterial membrane (see Fig. 4 in ref [27]). This transition implies a 90° rotation of each protomer within dimer I allowing the dimer to insert in the membrane. This exposes the hydrophobic peptide surface to the hydrophobic lipid tails whereas charged areas interact with the charged phospholipid head groups. Unfortunately, direct experimental support for this hypothesis is lacking.

A further hypothesis based on the results of electrochemical experiments proposes that the bacteriocin is able to form membrane pores some 7 Å in diameter [28].

Bacteriocin AS-48 selectively attacks bacterial membranes [29,30]. This observation is consistent with the general trend noted for some antimicrobial peptides and has been nicely illustrated by in vitro experiments using model membranes [31–33]. Bacterial membranes feature high proportions of anionic lipids such as dipalmitoyl posphatidyl glycerol (DPPG) or dipalmitoyl phosphatidic acid (DPPA) [34,35]. Experiments performed on AS-48 and DPPA monolayers have revealed that only at pH > 10 does energetically favourable interaction between these monolayers occur, but at the expense of substantial modification to the secondary structure of AS-48 [36]. This finding, however, contrasts sharply with the increased bactericidal effect detected at pH values between 4 and 8 in bioactivity experiments [37].

A number of experiments using engineered AS-48 derivatives have sought to identify the main amino acids responsible for the bioactivity

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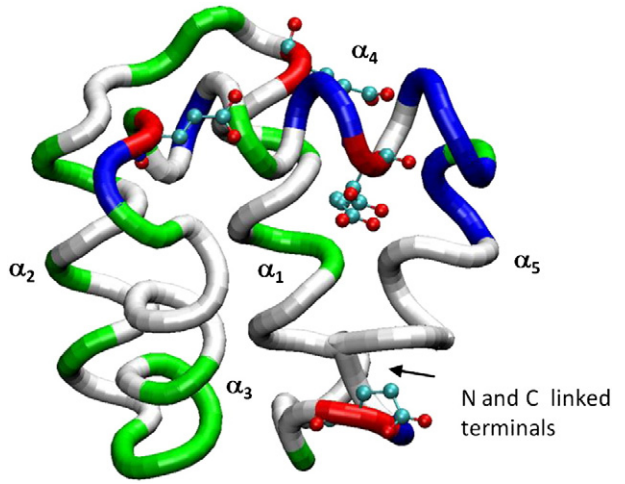

Fig. 1. Tube representation of the AS-48 protomer. Blue and red colours indicate positively and negatively charged residues, and green and white, polar and hydrophobic amino acids, respectively. Negatively-charged GLU resides are explicitly shown as the CPK model. The five helices α_1 to α_5 span residues 9–21, 25–34, 37–45, 51–62, and 64–5, respectively [26].

of this peptide [38–40]. The results of such studies indicate that wild type bacteriocin is the most active peptide [41].

The present study was designed to gain insight into the mechanism of action of bacteriocin AS-48 via a series of simulation experiments. For the simulations, we selected the MARTINI coarse-grained (CG) force field model since it is able to probe the spatial and time scales of systems beyond what is feasible using traditional all-atom models [42–44]. In effect, several studies have confirmed the consistency of different coarse-grained models used to simulate AMP systems and their interactions with lipid bilayers [6,45–50].

2. Computation methods

The starting structure for peptide dimer I was built directly from its X-ray structure (PDB ID: 1O83). For all the systems examined, the MARTINI CG force field model was used to map four atoms to one coarse-grained particle, i.e., four heavy atoms are represented by a single interaction centre [51,52]. DPPA lipid particles were similarly assigned to the DPPC molecule except that the particle associated with the choline group was removed leaving a net charge of −1 as the expected charge at physiological pH. Simulations were run in the NPT ensemble, coupling the system to a temperature and pressure bath according to the Berendsen coupling scheme [53]. The target temperature was 310 K and semi-isotropic pressure coupling was set to a reference pressure of 1 atm using a compressibility of 4.6×10^{-5} bar^{-1}. Lennard–Jones interactions were smoothly shifted to zero within the range of 0.9 and 1.2 nm and electrostatic interactions were also shifted to zero between 0 and 1.2 nm, according to recommendations for these coarse-grained simulations [48].

The total charge of the system was neutralized with 500 counter ions. Each protomer has a net charge of +6 and each DPPA lipid molecule has a −1 charge, as stated above.

The DPPA bilayer was built after the removal of choline particles from a previously equilibrated DPPC bilayer. This bilayer was duplicated in the X and Y directions, yielding a final system of 512 lipids. A water slab was added to the system to yield a Z dimension of 15.5 nm. This gives room for the later insertion of a peptide dimer, avoiding periodicity artefacts. The system was then equilibrated for 100 ns under the described conditions. In a subsequent step, the dimer was inserted in the water phase, and all water particles within 0.25 nm of any protein particle removed. The system was energy-minimized and then equilibrated using position restraints in protein pseudoatoms until lipid/protein, protein/water interaction energies had stabilized. In the next step, simulations of variable lengths were performed without further restraints. For systems with the protein inserted in the bilayer, the same procedure was applied. Protomers were placed in the selected position in the bilayer interior and lipid and water molecules within 0.25 nm of each protein particle were excluded from the model. Further details of the simulated systems are given below in the corresponding sections. The time-step used in all simulations was 0.03 ps, as recommended for MARTINI coarse-grained based simulations.

All calculations were performed using the GROMACS 4.5 package [54].

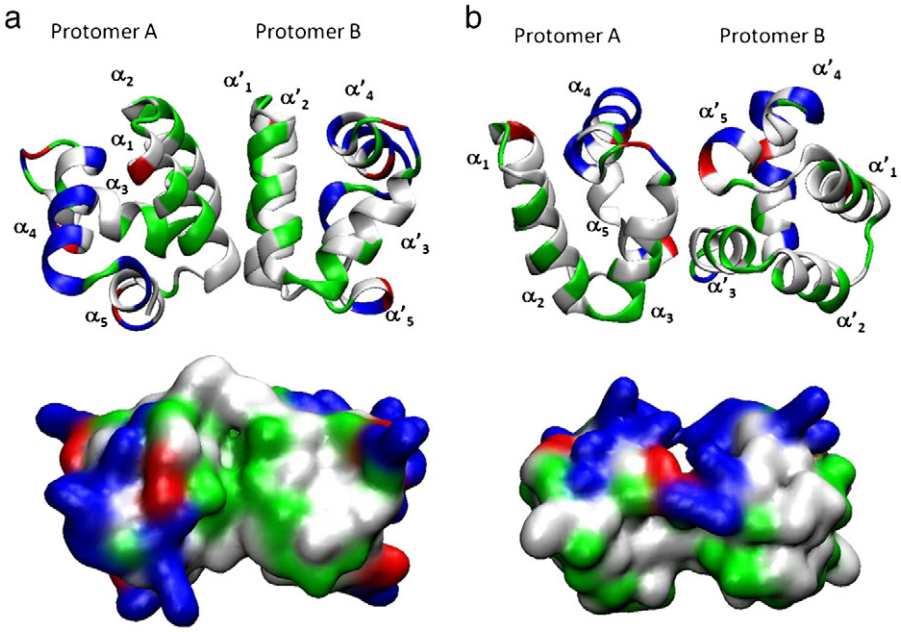

Fig. 2. The two dimeric forms of AS-48 indicated by crystallography. a: Dimeric form I (in aqueous solution, PDB ID: 1O83). b: Dimeric form II (in detergent, PDB ID: 1O84). Cartoon (top) and surface (bottom) representations in which the colours blue and red indicate positively and negatively charged residues, and green and white, polar and hydrophobic amino acids, respectively.