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Penetration of antimicrobial peptides in a lung surfactant model

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ABSTRACT

Molecular dynamics simulations were successfully performed to understand the absorption mechanism of antimicrobial peptides LL-37, CATH-2, and SMAP-29 in a lung surfactant model. The antimicrobial peptides quickly penetrate in the lung surfactant model in dozens or hundreds nanoseconds, but they electrostatically interact with the lipid polar heads during the simulation time of 2 μs. This electrostatic interaction should be the explanation for the inactivation of the antimicrobial peptides when co-administrated with lung surfactant. As they strongly interact with the lipid polar heads of the lung surfactant, there is no positive charge available on the antimicrobial peptide to attack the negatively charged bacteria membrane. In order to avoid the interaction of peptides with the lipid polar heads, sodium cholate was used to form nanoparticles which act as an absorption enhancer of all antimicrobial peptides used in this investigation. The nanoparticles of 150 molecules of sodium cholate with one peptide were inserted on the top of the lung surfactant model. The nanoparticles penetrated into the lung surfactant model, spreading the sodium cholate molecules around the lipid polar heads. The sodium cholate molecules seem to protect the peptides from the interaction with the lipid polar heads, leaving them free to be delivered to the water phase. The penetration of peptides alone or even the peptide nanoparticles with sodium cholate do not collapse the lung surfactant model, indicating to be a promisor drug delivery system to the lung. The implications of this finding are that antimicrobial peptides may only be co-administered with an absorption enhancer such as sodium cholate into lung surfactant in order to avoid inactivation of their antimicrobial activity.

1. Introduction

Cathelicidins have an important role against invasive bacterial infection in mammalian innate immune defense [1]. They exhibit a broad-spectrum of microbicidal activity against fungi, viruses, and bacteria [2,3]. Cathelicidins are cleaved into the antimicrobial peptide LL-37 by proteases [4]. The gene encoding LL-37 is a member of the human cathelicidin family. It is an antimicrobial peptide of 18 kDa found in lysosomes of polymorphonuclear leukocytes, keratinocytes and macrophages [5]. Chicken cathelicidin-2 (CATH-2) is an arginine-lysine rich peptide with two alpha-helical segments separated by a proline-induced kink which is essential for a strong broad-spectrum antibacterial activity [6]. SMAP-29 is a cathelicidin-derived 29 amino acid peptide deduced from sheep myeloid mRNA, which displays extremely high antimicrobial activity with a strong broad-spectrum antibacterial activity [7,8]. Cathelicidin acts together with defensins and high cathelicidin concentration are found in sites of inflammation, where they seem to be destroying microorganisms [9]. In general, antimicrobial peptides are also produced in the epithelium, suggesting that they are a type of primary defense [10]. Antimicrobial peptides have been suggested as future therapy for resistant bacteria to antibiotics due to its strong and broad spectrum antimicrobial activity with low development of resistance [5].

The bacterial membrane is damaged and punctured by cathelicidin. The general mechanism of cathelicidin involves the permeabilization of cell membranes of bacteria by the active cathelicidin [11]. Although cathelicidin may interact with DNA, protein, and cell wall synthesis, and protein folding, the interaction of cathelicidin with bacteria is electrostatic as the cationic charges of amino acids allow them to interact with the anionic surface of bacterial cell wall membranes [12]. However, cathelicidin may penetrate into the cell and bind important molecules to cell living [13]. It is not surprising that the exact mechanism of the antimicrobial peptide action is unknown [14,15].

Unfortunately, it has been recently discovered that the antimicrobial activities of the peptides CATH-2 and LL-37 disappears after intratracheal co-administration with lung surfactant in vivo [16]. There are none experiments on the antimicrobial activity of SMAP-29 after co-administration in vivo with lung surfactant. Although none experimental evidence is presented, the mechanism of the deactivation is suggested to be the electrostatic interaction

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of peptides with the lung surfactant, avoiding the contact between peptides and bacteria cell wall. The main strategies to overcome this barrier are to use liposomes, bile salts, non-ionic surfactants or fatty acids as absorption enhancers for pulmonary drug delivery [17]. The encapsulation of antimicrobial peptides using bile salts seems to be the first choice because bile salts dramatically enhance the alveolar absorption of drugs, do not affect gas exchange or lung compliance, and are the most common absorption enhancers used to enhance drug delivery to the lung [17]. On the other hand, to the best of our knowledge, the interaction of antimicrobial peptides encapsulated with bile salts (or even antimicrobial peptides alone) on lung surfactant models has not been investigated theoretically in order to understand the molecular mechanism of absorption in the lung [5,6,8].

The coarse-grained molecular dynamics (CGMD) of the adsorption of nanoparticles on lung surfactant models has been studied in the literature [18–26]. The CGMD seems to be a very useful tool that has been used for modeling of lung surfactant models in order to understand the conformational and dynamic information of the system with different surface tensions to mimic the changes on surface tension during the respiration process. Furthermore, antimicrobial peptides interacting with phospholipid bilayers have been extensively studied by CGMD simulations. [27–41] Many aspects on membrane poration, [27–31] buckling, [35] and penetration [33,36] as well as energetic considerations [32,36,37,40] with coarse-grained simulations are discussed. However, to the best of our knowledge, they have not been investigated as interacting with phospholipid monolayers and/or bile salts. Up to date, the existing CGMD simulations related to bile salts are about aggregation in aqueous solution, [42] formation of micelles, [43,44] interaction with phospholipids [45] and carbon nanotubes [46]. The analysis of the CGMD simulations, such as area per lipid, orientation, order, and diffusion of lipids can be drawn, but more important, it is possible to observe the monolayer collapse, nanoparticle permeation, bile salt spreading, and antimicrobial peptide delivery.

This study provides information on the absorption of antimicrobial peptides LL-37, CATH-2, and SMAP-29 into mixed phospholipid monolayers composed of 41% 1,2-dipalmitoyl-sn-*glycero*-3-phosphocholine (DPPC), 36% 1-palmitoyl-2-oleoyl-sn-*glycero*-3-phosphocholine (POPC), 10% 1-palmitoyl-2-oleoyl-sn-*glycero*-3-phospho-(1′-rac-glycerol) (sodium salt) (POPG), 5% 1-palmitoyl-2-oleoyl-sn-*glycero*-3-phosphoethanolamine (POPE), and 8% cholesterol (CHOL) using coarse-grained MD as a lung surfactant model [47]. The surfactant proteins, which represent less than 2% of exogenous natural lung surfactants, are eliminated from the model in order to simplify the simulation. The bile salt sodium cholate is used to encapsulate the antimicrobial peptides and enhance its absorption in the lung surfactant model. The chemical structures of these compounds are shown in Fig. 1S. The main aim of this study is to investigate the use of bile salts as absorption enhancer of antimicrobial peptides in a lung surfactant model.

2. Methodology

2.1. Parameters and model

The computational package GROMACS 5.0.6. was used to perform the CGMD simulations. [48] The coarse grained model parameters for DPPC, POPC, POPG, POPE, cholesterol, sodium cholate, and water were standard and obtained from the literature. [27,49–51] The water model groups four water molecules. All molecules were represented by coarse grained particles that group around four heavy atoms together, which are standard components of this force field. The framework representations of the coarse grain models for DPPC, POPC, POPG, POPE, CHOL, and sodium cholate are described in Fig. 2S. The types of beads are shown in supplementary material after Fig. 2S. Fig. 3S shows the representation of coarse grain models for the peptides LL-37, CATH-2, and SMAP-29.

The script MARTINIZE was used to generate the peptide topology and structure files based on an atomistic structure file. The lung surfactant model (DPPC/POPC/POPG/POPE/CHOL) was built with 1024 molecules of lipids each monolayer using the script INSANE [49]. Initially, using the program g_editconfig, the bilayer system was split in two individual monolayers by 6 nm. Using the program g_solvate, it was built a water box with dimensions of 25 nm x 25 nm x 6 nm in order to generate a water slab with around 29,000 water molecules. The density was set to 1 kg L^{-1}. Then, the water slab was inserted between the two lipid monolayers, allowing a vacuum slab of 15 nm on the top and another on the bottom of lipids. The lipid plane was set parallel to the XY plane of the system, and the lipids were oriented with the polar heads directed towards to the water phase. The whole system was placed in a space filing box of 25 nm x 25 nm x 40 nm replicated periodically in all directions, x, y, and z [26]. A molecule of antimicrobial peptide (or the encapsulated one) was placed in the vacuum space in a random manner very close to the air/lipid interface. The encapsulated peptide was built as follows. The peptides CATH-2 and SMAP-29 were positioned in the middle of a 7 nm x 7 nm x 7 nm box, which was randomly filled with 150 molecules of bile salt around the peptide molecule. As the peptide LL-37 is elongated, it was positioned in a 9 nm x 7 nm x 7 nm box and the 150 molecules of bile salt were randomly added around it. After building the lipid monolayers, the g_genion program was used to generate ions by replacing water molecules of the box. Analogously, after placing the peptides or nanoparticles, water molecules on the box were replaced by ions. Those ions were Na+ and Cl-, always used to neutralize the system as a whole. Then, the system was equilibrated using the procedure below and the peptide/bile salt cluster was inserted close to the air/lipid interface. Fig. 1 shows the starting systems for the peptide absorption (Fig. 1A–C) and for the peptide/bile salt penetration (Fig. 1D–F). The systems were visualized with the VMD computer program [52]. The amino acid sequences for LL-37 are LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES (pdb code: 2k6o), for CATH-2, LVQRGRFGRFLRKIRRFRPKVTITIQGSARF (pdb code: 2gdl), and for SMAP-29, RGLRRLGRKIAHGVKKYGPTVLRIIRIAG (pdb code: 1fry).

2.2. Molecular dynamics

Before all CGMD simulations, the steepest descent method was used to minimize the energy of the system until it reaches 100 kJ mol^{-1} nm^{-1} in order to eliminate atoms overlapped during the system preparation. The time step of 20 fs was used in all simulations. The relative dieletric constant was the default value for this force field, 15 [53]. The cut-off for long-distance interactions in the particle mesh Ewald(PME) summation [54] was 1.0 nm. The cutoffs for Lennard-Jones interactions were shifted to zero between 0.9 and 1.2 nm and Coulomb interactions were shifted to zero between 0 and 1.2 nm, which are the standard cutoffs for the coarse grained force field. The neighbor list for non-bonded interactions was updated every 10 steps.

The system was further equilibrated with a simulation time of 100 ns using the leap-frog algorithm. [55] This step gave more flexibility to water molecules in order to allow more freedom for them to interact with lipid head groups. A production CGMD run with a simulation time of 2 μs was done using the leap-frog algorithm [55]. The last microsecond of the trajectory was used in order to average the properties of interest. Water, peptides, sodium cholate, and lipid molecules were coupled separately using the v-scale thermostat [56] with a time constant of 1 ps at 310 K. The compressibility