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Effect of physicochemical properties of peptides from soy protein on their antimicrobial activity

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ABSTRACT

Antimicrobial peptides (AMPs) kill microbial cells through insertion and damage/permeabilization of the cytoplasmic cell membranes and has applications in food safety and antibiotic replacement. Soy protein is an attractive, abundant natural source for commercial production of AMPs. In this research, explicit solvent molecular dynamics (MD) simulation was employed to investigate the effects of (i) number of total and net charges, (ii) hydrophobicity (iii) hydrophobic moment and (iv) helicity of peptides from soy protein on their ability to bind to lipid bilayer and their transmembrane aggregates to form pores. Interaction of possible AMP segments from soy protein with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine/1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPC/POPG) bilayers, a mimic of bacterial cell membrane, was investigated. Pore formation was insensitive to helicity and occurred for hydrophobicity threshold in the range of −0.3–0 kcal/mol, hydrophobic moment threshold of 0.3 kcal/mol, net charge threshold of 2. Though low hydrophobicity and high number of charges help in the formation of water channel for transmembrane aggregates, insertion of peptides with these properties requires overcome of energy barrier, as shown by potential of mean force calculations, thereby resulting in low antimicrobial activity. Experimental evaluation of antimicrobial activity of these peptides against Gram positive *L. monocytogenes* and Gram negative *E. coli* as obtained by spot-on-lawn assay was consistent with simulation results. These results should help in the development of guidelines for selection of peptides with antimicrobial activity based on their physicochemical properties.

1. Introduction

Antimicrobial peptides (AMPs) kill microbial cells through insertion and damage/permeabilization of the cytoplasmic cell membranes and has applications in food safety and antibiotic replacement [1–4]. Computer simulation is a useful tool to investigate the interaction of peptides and lipid membrane [5–9]. Pore formation on cell membrane is an indication of antimicrobial activity for AMPs, and the formation of pores is accompanied with water channel formation and bending of lipid heads [1]. The types of AMPs that results in pore formation on cell membrane can be varied based on their mechanisms of action, structure, mode of interaction with membrane, etc. [10]. For examples, AMPs can be classified as linear cationic antimicrobial peptides (LCAP), cationic peptides stabilizing structure by interchain covalent bond (C-CP), peptides rich in proline and arginine (PRP), etc. [11]. However, AMPs share some common properties, for instances, they are short peptides (normally 10–50 residues) [12,13], with high hydrophobicity which is required for lipid binding [14]. The higher amount of net charges can result in more interaction with anionic membrane [14]. Also it has been shown that many AMPs adopt an α helical secondary conformation in the hydrophobic environment of lipid bilayer [15].

The prediction of antimicrobial activity of a peptide should consider a combination of physicochemical properties such as size, charge, hydrophobicity, residue composition and conformational features such as secondary structure and amphiphilic character. The size, charge and hydrophobicity of a peptide can be calculated and quantified; also the amphiphilic character and secondary structure can be inferred from hydrophobic moment [16]. Positive net charge could lead to a favorable peptide-membrane binding, since the cell membrane of bacteria are normally negatively charged, resulting in an electrostatic interaction [17]. High number of charges, however, would also result in difficulty in penetration of peptides from membrane surface to interior, because of favorable water-peptide interaction and phospholipid heads-peptide interaction [18]. Moreover, the confinement of charges within

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the pore can facilitate a higher energy barrier for pore formation [19–21]. As a result, the number of charges for a potential AMP is expected to be within an acceptable range. Peptides with high hydrophobicity would encounter a smaller free energy barrier when they penetrate the bilayer from aqueous to lipid environment [14]. However, sufficiently large hydrophobicity can cause peptides to aggregate in the aqueous environment [16]. Therefore, naturally occurring AMPs are found to have hydrophobicity in a moderate range [22]. The distribution of hydrophobic and hydrophilic residues determines the amphipathicity of a peptide [23]. Alternating sequence of hydrophobic and hydrophilic residues along the peptide backbone will result in orientation of hydrophobic groups outwards into the lipid environment and facilitate formation of water channel by its aggregate with the hydrophilic groups pointing inwards [24]. The distribution of hydrophobic and hydrophilic residues can be deduced from the hydrophobic moment [25].

AMPs can be effective against drug resistant bacteria and can therefore find application antibiotic replacement in human health and in animal feed. AMPs can also be immobilized on food packaging films for food safety applications. Soy protein is an attractive, abundant natural source for commercial production of AMPs. In our previous research, we have developed a methodology to predict antimicrobial activity of peptide segments from soy protein subunits β-conglycinin and glycinin [26]. The prescreening of possible antimicrobial peptides from soy protein were based on their physiochemical properties such as hydrophobicity, charges and hydrophobic moment. Peptide segments that fulfilled the selection criteria based on these properties were further investigated for their antimicrobial properties using molecular dynamics (MD) simulation. Surface binding of peptide onto lipid bilayer and water channel formation by aggregate of transmembrane peptides were considered to be two essential factors to predict antimicrobial activity in MD simulation. However, there is a lack of information on the thresholds of hydrophobicity, hydrophobic moment and charge for water channel formation and lipid head bending. Such an information is essential for rational selection of AMP segments from a protein. This manuscript investigates the effects of different parameters on antimicrobial activity and quantifies thresholds on charge, hydrophobicity and hydrophobic moment for antimicrobial activity of these peptides that are of 20–30 residues in length.

2. Materials and methods

2.1. Materials

Identified peptide segments were synthetized, purity > 95% (GenScript, USA). *L. monocytogenes F4244* and *E. coli O157:H7 EDL933*, Trifluoroacetic acid (TFA) and Dimethyl sulfoxide (DMSO) were from Sigma-Aldrich.

2.2. Selection of peptide segments from soy protein subunits 11S and 7S

Peptide segments were selected from five subunits from 11S: G1 (Uniprot: P04776), G2 (Uniprot: P04405), G3 (Uniprot: P11828), G4 (Uniprot: P02858) and G5 (Uniprot: P04347) and three subunits from 7S: α (Uniprot: P13916), α' (PDB:1UIK) and β (PDB:1UIJ) with 20–30 amino acids in length. We investigated the effects of different number of net charge (number of positive charges minus number of negative charges) and total charge (sum of number of charges (irrespective of positive or negative)), Eisenberg consensus scale hydrophobicity and hydrophobic moment [27]. The physical properties of these peptides that are investigated are given in Table 1.

2.3. Molecular dynamics (MD) simulation in lipid bilayer system

The MD simulation method is adapted from Xiang et al. [26]. Briefly, the initial lipid bilayer structure for the simulation was built using the CHARMM-GUI web tool [28]. The bilayer in the simulation was represented by mixed 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC, neutral)/2-oleoyl-1-pamlitoyl-sn-glyecro-3-glycerol (POPG, negatively charged) in a 3:1 ratio bilayer. POPC/POPG mixed bilayers are negatively charged membrane, mimicking a bacterial membrane. The peptide/lipid ratio was 4/128 with four transmembrane peptides inside the lipid system. 0.15 M KCl was added to neutralize the system. All-atom MD simulation was employed with Amber14 software package [29]. The snapshots were visualized using VMD [30] software. The secondary structural propensities was calculated using the DSSP method [31]. The peptide initial structures were obtained from implicit MD simulation after 50 ns using AMBER14 software and AMBER ff99SBildn force field [32], with a dielectric constant of 20, which mimics the lipid environment.

2.4. MD simulation using CHARMM36 force field

The results of simulation using CHARMM36 force field are presented here. Comparison of these results with those from simulations using Amber force fields are presented in the *supporting information*. The simulation procedure is adapted from Xiang et al. [26]. The system was slowly heated to 303° K after minimization. The protein-membrane system was then relaxed prior to running production MD to reach the target temperature (303 K). Molecular dynamics simulation in the constant volume (NVT) ensemble was carried out until the target temperature of 303° K was reached. Constant pressure (NPT) ensemble was used in the system simulation. Subsequent simulation was carried out at 303° K up to 200 ns with a time step of 2 fs. Langevin dynamics was employed with a collision frequency $\gamma = 1\ ps^{-1}$. Pressure was maintained at 1 bar using semi-isotropic pressure with the Berendsen barostat. Periodic boundary simulations based on the particle mesh Ewald (PME) method was carried out using NPT method with a cutoff at 8 Å.

2.5. Umbrella sampling simulation

Umbrella sampling simulation was employed for calculating the transfer free energy of a peptide from water to bilayer surface and from the surface to the interior of the bilayer. The reaction coordinate is chosen to be the z coordinate of the distance between the center of mass of the whole peptide being pulled, and the center of mass of the lipid bilayer. The sampling calculation regions was from 30.9 Å (peptide in water) to −15.1 Å (peptide on the lower lipid leaflet) along the z coordinate (perpendicular to the bilayer). To make sure the whole regions along the coordinate would be sampled, the force constant for the umbrella potential was setup as 20 kcal/mol/Å^2 and the window interval at 0.4 Å. Calculations for each window were performed for 120 ns per window except the bottom ten windows (−11.1 to −15.1 Å) for which the calculations were extended to 130 ns per window. The initial structures for the umbrella sampling simulation was setup by placing the crystal structure of the peptide with center of mass at the window position, and by running minimization, equilibration and production of the system. During simulations, all the ensembles, cutoff, temperature and surface tension were maintained the same as described in MD simulation using AMBER force field (Supporting Information). The free energy profile was evaluated using weighted histogram analysis method (WHAM) [33].

2.6. Bioassay and viability studies

The potential candidates of peptide fragments from soy protein for antimicrobial activity that were identified using MD simulation were commercially synthesized (GenScript, USA). The spot-on-lawn method with serial dilution procedure is adapted from Xiang, et al. [26]. Antimicrobial activity of synthesized peptide (SP) fragments was tested against *L. monocytogenes F4244* (Gram positive) and *E. coli O157:H7*