4P079

Structure and Stability of the Binding of HNP-1 with DPPC Lipid Bilayer: A Molecular Dynamics Study

(Kanazawa Univ.*, Bandung Institute of Technology**) Nandia Primasari*,**, Hiroaki Saito*, Santi Nurbaiti**, Kazumoto Kawaguchi*, Hidemi Nagao*

[Introduction] Defensins are cationic immune peptides contain three disulfide bonds, which are in charge of protecting from pathogenic microbes. Mammalian defensins are classified into α, β, and θ category, based on the size and the disulfide bond pattern. HNPs 1, 2, and 3, are the most abundant forms of human α-defensins. From many experimental studies, it is known that HNP-3 is less microbicidal than HNPs 1 and 2 against most of tested microbes. HNP-1 and HNP-2 showed almost indistinguishable activities against the gram-positive strains, however HNP-2 appeared to be more potent than HNP-1 against the gram-negative strains. It leads to the research of the interaction of HNP-1 with lipid membrane.

It is known that HNP-1 can kill the pathogen by disrupting its membrane. This mechanism of the membrane disruption by the HNP-1 has been predicted in some studies. The details of bind structure, molecular interactions, and the stability of the HNP-1, which are key properties to understand the mechanism, have not been clarified yet. In this study, we thus carried out the molecular dynamics (MD) simulations of the DPPC lipid bilayer in the presence and absence of HNP-1 dimer and analyzed the dynamical structure of the system in detail. The effects of HNP-1 binding on the membrane structure and the molecular affinity between the polar headgroups of the membrane and the arginine side of HNP-1 were also investigated in this study.

[Experiment] MD simulations were carried out by using NAMD 2.9 and VMD 1.9.1 simulation package. CHARMM36 force field and TIP3P water model were adopted for lipid and water molecule, respectively. A structure of the HNP-1 was obtained with X-ray diffraction experiment and was downloaded from the Protein Data Bank (3GNY) as the initial structure. The initial structure of DPPC (dipalmitoylphosphatidylcholine) membrane contains 128 lipid molecules and 4000 water molecules, and this was constructed by using Packmol.

Figure 1 shows the predicted membrane disruption mechanism of the HNP/membrane [1]. This model reference [1] shows the process when the arginine side of HNP initially binds to the bilayer surface electrostatically. This process is a part of the defensins-mediated pore formation.

In this study, we modeled the binding structure, which the HNP-1 dimer is placed on the equilibrated membrane using VMD, and investigated the dynamical structure of the HNP-1/DPPC membrane by the MD simulation. First, we carried out the MD simulation of the DPPC membrane in the absence of HNP-1 dimer for 50 ns. Langevin thermostat and barostat were used to control the system temperature (T=323.15K) and pressure (P=1atm), and the oscillation period of thermostat and barostat were 5 ps and 0.2 ps, respectively. After that, we analyzed the membrane structure parameters: area per lipid

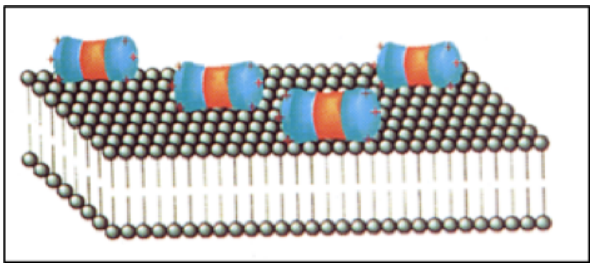

Figure 1. Model reference of HNP-1 bound to DPPC lipid bilayer (Reference [1])

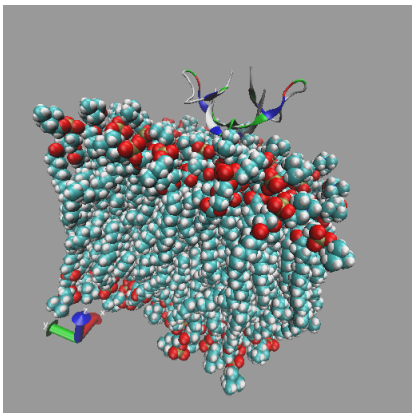

Figure 2. HNP-1 dimer binds to the DPPC bilayers

molecule ($A(t)$); membrane thickness; and order parameter ($S_{CD} = \frac{1}{2}\langle 3\cos^2\theta - 1\rangle$), to get the appropriate initial condition for the system simulation and see the difference with the condition when HNP-1 exists. Furthermore, we construct the system by placing the HNP-1 on the equilibrated membrane (Figure 2). Then, we carried out MD simulation of this system at 320 K through four stages: melting the lipid tails, minimization and equilibration with protein constrained, equilibration with protein released, and the NPT long production run for 50 ns. The structural stability of the HNP-1 was analyzed by estimating the RMSD, RMSF, and SASA of the peptide.

[Results and Discussion] The membrane quantities of the DPPC bilayers are evaluated by using the area per lipid molecule, membrane thickness, and the order parameter. The effect of binding of HNP-1 on the membrane interface was accessed by these membrane quantities in the absence and presence of the HNP-1. In the absence of HNP-1, the simulation yielded $\langle A\rangle = 61.16$ Å^2 /lipid. This result is close to the experimental data, $\langle A\rangle = 63.0 \pm 1.0^{87}$ Å^2. The bilayer thickness is found to be 38.8 Å, being agree with the experimental value of 38.3 Å.

The equilibration MD simulations of the DPPC membrane in the presence of HNP-1 have been done for 1 ns. Figure 3 shows the RMSD as a function of MD time steps. We found that the structure of HNP-1 could reach a stable position at the interface quickly and oscillates around 1.5 Å. This indicates that the structure of HNP-1 on the membrane interface is sufficiently maintained. Further results and analysis will be displayed in the poster session.

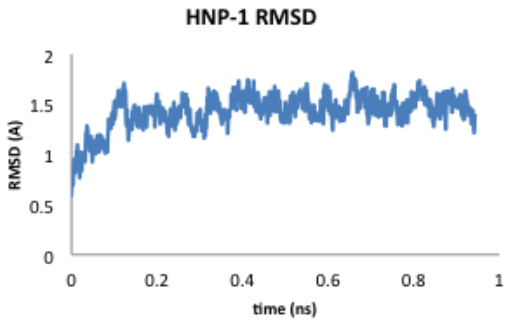

Figure 3. RMSD of HNP-1 at the equilibration with protein released stage for 1 ns

[References]

[1]. Michael E. Selsted and Andre J. Ouellette. *Defensins in granules of phagocytic and non-phagocytic cells*. Elsevier, Trends in Cell Biology Vol. 5, March, 1995.

[2]. Jeffrey B. Klauda, Richard M. Venable, J. Alfredo Freites, Joseph W. O'Connor, Douglas J. Tobias, Carlos Mondragon-Ramirez, Igor Vorobyov, Alexander D. MacKerell, Jr., Richard W. Pastor. *Update of the CHARMM All-Atom Additive Force Field for Lipids: Validation on Six Lipid Types*. J. Phys. Chem. B 2010, 114, 7830-7843.