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Molecular dynamics simulation of c-ring of F-type ATP synthase in explicit membrane

○Kyoko Nakajima, Sotaro Fuchigami, Mitsunori Ikeguchi, Akinori Kidera
Yokohama City Univ, Internat. Graduate School of Arts and Sciences,
Supramolecular Biol.

F-type ATP synthase is a rotary nanomachine that synthesizes ATP from ADP using electrochemical gradient of proton or Na⁺ ion. It consists of two components, water-soluble F₁ and transmembrane F₀. The major component of the F₀ unit is composed of 10-15 c-subunits, called c-ring, having hourglass-like shape with a central cavity. It was observed in the c-ring structure without F₁ unit that the cavity was filled with phospholipids making a plug to maintain electrochemical gradient. However, atomistic details of the plug, such as the number of lipids forming the plug and their positions and conformation in the cavity, have not been clarified. In this study, we investigated the structure and dynamics of c-ring with the plug by molecular dynamics (MD) simulation. We constructed a system in the explicit membrane environment as follows. First, the crystal structure of c-ring from *Ilyobacter tartaricus* (PDB ID: 1YCE) was embedded in a lipid bilayer of POPC by excluding a suitable number of lipid molecules. Next, as the plug, 4 and 2 lipids were inserted manually into the periplasmic and cytoplasmic sides, respectively, according to the volume of the central cavity. Consequently, the system consisted of about 150,000 atoms. Simulations were performed by the MD program system, MARBLE, using the force field parameters of CHARMM27/CMAP. After energy minimization, equilibration and product runs were carried out in the NPT ensemble. We will discuss stability and dynamical properties of c-ring and the plug lipids.

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Attachment of yellow fluorescent protein to influenza hemagglutinin at cytoplasmic tail inhibits membrane fusion activity of hemagglutinin

○Mari Gotoh, Masae Takashima, Tomoko Okada, Yoshikatsu Ogawa
National Institute of Advanced Industrial Science and Technology (AIST)

Hemagglutinin (HA) is one of the major transmembrane proteins of influenza virus, which causes viral uncoating via its membrane fusion activity. HA is composed of disulfide-linked HA1 and HA2 subunits. HA1 binds to viral receptors on target cells and HA2 anchors the HA into the viral membrane through single transmembrane domain (TM). HA2 cytoplasmic tail (CT) consists only of 11-amino acids. Deletion of CT does not significantly effect on the fusion, whereas by replacing the TM and CT with a glycosylphosphatidylinositol anchor (GPI) the protein could induce only hemifusion. On the other hand, the effect of terminally-extended CT is of our interest. In this study, we have cloned the HA cDNA from influenza A/Hong Kong/1/68 (H3N2) strain and constructed a fusion-protein with yellow fluorescent protein (YFP) at the C-terminal CT of HA2. HA-YFP was expressed on the surface of HeLa cells and membrane fusion activity of the protein was examined after trypsin and neuraminidase treatments of the cells. The HeLa cells expressing HA-YFP were bound to the U937 cells, which were dual-stained with a fluorescent lipid probe, R18, and a fluorescent fluid-phase probe, calcein. Then their fusion was analyzed by confocal microscopy under fusion-competent conditions at low pH. The result showed absence of lipid mixing and content mixing between the HeLa and the U937 cells. The inhibition of fusion activity of HA by the attachment of YFP to CT of HA2 will be a useful experimental model for the studies of intermediate steps of membrane fusion.

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Molecular Dynamics Simulations of Mechano-Gating in the *E-coli* Mechanosensitive Channel MscL

○Yasuyuki Sawada¹, Masaki Murase², Masahiro Sokabe^{1,2,3}

¹Dept. Physiol., Nagoya Univ. Grad. Sch. Med, ²ICORP/SORST Cell Mechanosensing, JST, ³Dept. Mol. Physiol., NIPS, NINS

The bacterial mechanosensitive channel of large conductance, MscL, is constituted of homopentamer with two transmembrane α -helices and gated directly by membrane tension. The 3D structure of MscL from *Escherichia coli* has been proposed based on the crystal structure of its homologue from *Mycobacterium tuberculosis* and certain information on MscL conformational changes during opening have been provided. However, it is unclear where in the structure of MscL can sense membrane tension and how the sensed force can induce channel opening due to a lack of information on MscL-lipid interactions. To address these problems, we constructed a model, in which a closed MscL was embedded in a lipid bilayer, and performed MD simulations. Membrane tension was generated by reducing the lateral pressure in the membrane resulting in a tension profile with two peaks around the glycerol moiety of the lipid in the outer and inner leaflets, respectively. Upon tension application, transmembrane α -helices were gradually tilted by the dragging force from the bilayer, leading to channel opening. To evaluate our system, we modeled a F29N mutant which opens more easily than wild type and confirmed that it reproduced experimentally observed behavior. Results suggest that MscL senses membrane tension at its transmembrane portion near the lipid-water interface in the outer leaflet. The sensed forces will tilt down the rigid α -helices towards the membrane plane accompanied by sliding of interacting points between the helices of subunits, resulting in channel opening.

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The Importance of Single Residue in Retinal Binding Pocket for Color-tuning and Stability in Microbial Rhodopsins

○Keon Ah Lee, Ah Reum Choi, Sa Ryong Yoon, Kwang-Hwan Jung

Dept. of Life Science and Interdisciplinary Program of Integrated Biotechnology, Sogang University

Many microbes have retinal-binding integral membrane proteins called microbial rhodopsins that have two distinct functions: sensory transduction and ion transport. One of the retinal binding sites in *Anabaena* Sensory Rhodopsin (ASR) which found in cyanobacteria is Gly135. According to primary sequence comparison of microbial rhodopsins, the residue at this position is conserved among other microbial rhodopsins at least 3 amino acid: Gly, Ala, and Ser: G155 for proteorhodopsin (PR), S181 for *Gloeobacter violaceus* rhodopsin (GR), S151 for *Guillardia theta* rhodopsin, and G130 for *Natronomonas pharaonis* rhodopsin (NpSRII). In order to understand the importance of the G135 in ASR, we constructed, expressed, and purified 19 other mutated ASR and measured the absorption spectra. Among these, 5 mutants (G135S, G135C, G135A, G135T, & G135M) were able to purify as a colored-pigment which were all showed the red-shifted λ_{max} . Moreover, the site-directed substitution G155S and G155A in PR, S181G and S181A in GR, S151G and S151A in *Guillardia* rhodopsin, and G130S and G130A in NpSRII shift λ_{max} of each to the red. We are further investigating what other effects on the function of microbial rhodopsin when the residue is substituted by corresponding amino acids. For this purpose, we are performed photocycle of all mutants, proton pumping activity, and pKa values of PR and GR.