Protein-phospholipid interplay in a Ca^{2+}-pump revealed by X-ray solvent contrast modulation

It is well established that transmembrane helices of a membrane transport protein may rearrange substantially during the reaction cycle. In fact, some transmembrane helices in the Ca^{2+}-pump (Ca^{2+}-ATPase or SERCA1a) undergo large scale rearrangements [1], involving apparently perpendicular movements of 10 Å to the bilayer plane during the reaction cycle. If that occurs without bringing residues in/out of the bilayer, the bilayer has to be extremely flexible. Yet, in the other part of the reaction cycle, that is, in releasing bound Ca^{2+} to the lumen of the sarcoplasmic reticulum, a 100° rotation of the A domain is converted to the downward movement of the M4 helix, which requires that the bilayer does not largely deform [1]. Thus, the bilayer exhibits an apparently contradictory nature at different stages of the reaction cycle [2]. To resolve this problem we ought to visualize the lipid bilayer in the crystals of the Ca^{2+}-pump.

Our knowledge on the protein-phospholipid interaction (Fig. 1) was severely limited [3] because conventional X-ray crystallography failed to resolve phospholipids unless they are immobilized between protein molecules in the crystal lattice. This is because phospholipids are extremely mobile and contribute to only low resolution structure factors discarded in conventional crystallography, although the actual contribution of the bilayer exceeds that of protein at lower than 15 Å resolution [2]. This means that the phases obtained from protein atomic model cannot be correct and we ought to develop an experimental method for obtaining phases for such low resolution reflections.

By developing X-ray solvent contrast modulation and collecting diffraction data for all reflections from 170 to 3.2 Å resolution at SPring-8 **BL41XU** [2], we have succeeded in visualizing the entire first layer phospholipids (*ca.* 45 molecules) that surround the transmembrane region of the Ca^{2+}-pump crystallized in four different states and in refining the atomic models (Fig. 2 and Fig. 3). The atomic models were further examined by molecular dynamics simulations (Fig. 3(b)). Several key findings in this study are: (i) The bilayers are far from flat (Fig. 2(a)) and change their thickness (30.9–33.4 Å; Fig. 3), the number of the first layer phospholipids (44–48) and, accordingly, the cross-section of the transmembrane region of the protein during the reaction cycle. These differences will cause substantial changes in free energy and may work as driving forces to proceed the reaction. (ii) Phospholipids are anchored primarily by Arg/Lys-phosphate salt bridges (Fig. 1) and follow the movements of transmembrane helices, but they are less than half of the first layer phospholipids. There are apparently two classes in anchoring basic residues. The first one consists of those “snorkelling” from within the bilayer (Fig. 1 left). They cause local distortion of the bilayer when the transmembrane helices move (e.g. R63 and K262; Fig. 2(a)). Such distortion will act as a counter force to bring the transmembrane helices back to the most stable positions but may even be used as an energy source for rearranging them. (iii) The second class of basic residues (Fig. 1 right) extend their side chains through the cytoplasm to exploit phospholipids as “anchors” for conformational switching (e.g. R324

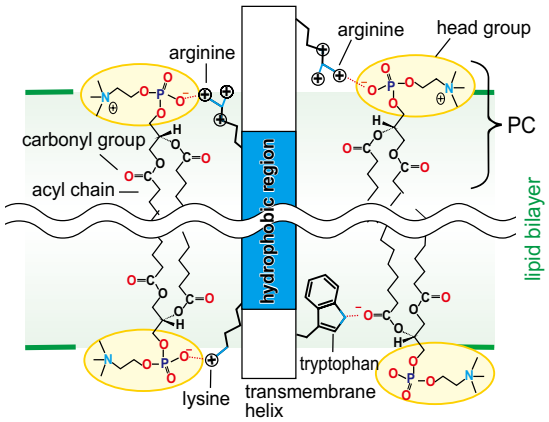

Fig. 1. Schematic illustration to show polar interactions between protein residues and phospholipids (phosphatidylcholine (PC) in this case) that constitute a lipid bilayer. The acyl chain of phospholipid varies between 14-20 carbon chain. The thickness of the bilayer can be affected by these residues, causing “protein-lipid mismatch” [3].

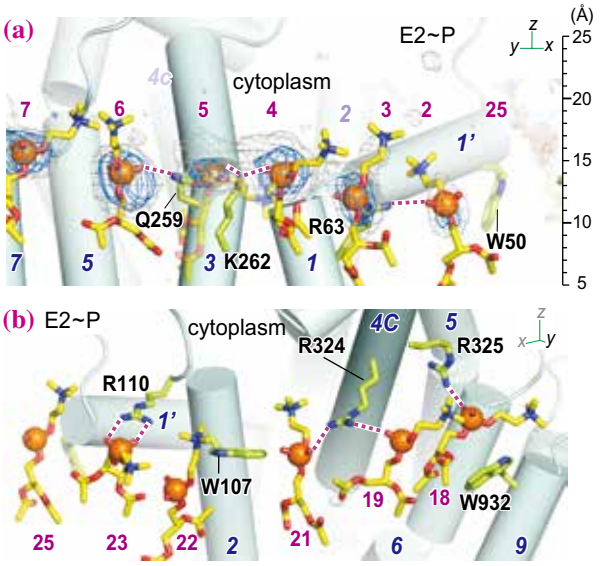

Fig. 2. First layer phospholipids that surround the transmembrane region of the Ca^{2+}-pump. Atomic models of phospholipids (from the head group to the carbonyl group of phosphatidylcholine; sticks with orange spheres for phosphorous atoms) are based on electron density maps derived from X-ray solvent contrast modulation. Blue and gray nets in **(a)** represent $2|Fo|-|Fc|$ electron density maps calculated at 3.2 Å resolution with combined phases and contoured at 0.7σ (gray) and 1.0σ (blue). Cylinders represent α-helices. Small numbers in purple identify phospholipids, and those in italics transmembrane helices (M1-M10). Purple broken lines represent likely hydrogen bonds. The ruler in the margin of **(a)** shows the distance from the bilayer center.

in Fig. 2(b)). (iv) The inclination of the entire protein changes during the reaction cycle (Fig. 3) governed primarily by a belt of Trp residues, acting as membrane "floats". Such change in inclination is a mechanism to allow large perpendicular movements of transmembrane helices.

Thus, we now see that phospholipids are a key component of calcium pump function and phospholipid-Arg/Lys and phospholipid-Trp interactions have distinct functional roles in the dynamics of ion pumps. We certainly expect similar protein-phospholipid interplay in other membrane proteins.

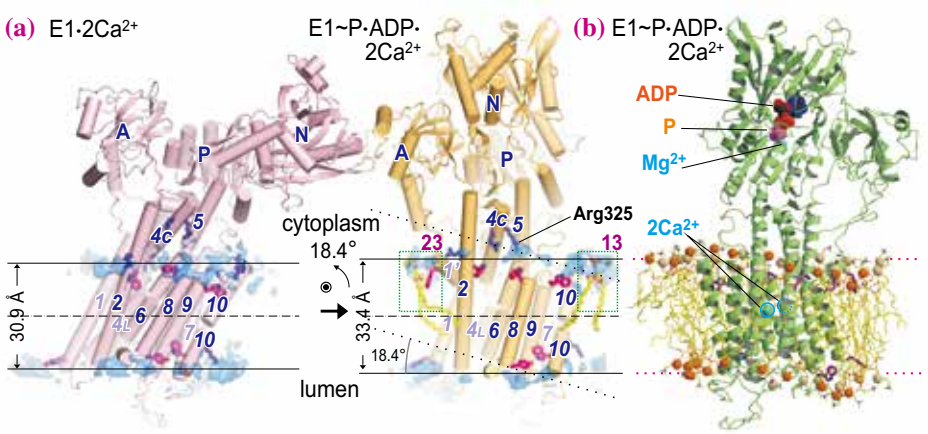

Fig. 3. **(a)** A change in orientation of the entire Ca^{2+}-pump molecule (18.4°) caused by the binding of ATP and transfer of the γ-phosphate to the pump protein. Placed so that the planes approximating the positions of the phosphorous atoms (horizontal solid lines) become horizontal in each crystal form. Inclined dotted lines show the corresponding planes in the conventional alignment with the M7-M10 helices, which do not undergo a rearrangement in the reaction cycle. Cyan surfaces show contrast modulation electron density maps for bilayers calculated at 4.5 Å resolution and contoured at 0.44 $e^{-}/Å^3$. Atomic models for a phospholipid molecule (PL23) around M1', an amphipathic helix, and that (PL13) near M10 in the cytoplasmic leaflet are shown in sticks (also boxed) in E1~P·ADP·$2Ca^{2+}$. **(b)** A full atomic model of the Ca^{2+}-pump and the first layer phospholipids after a 100 ns molecular dynamics simulation, viewed from the opposite direction to that in **(a)**. Orange spheres represent phosphorous atoms in the phospholipid head groups. The side chains of Arg/Lys and Trp/Tyr appear as dark blue and magenta sticks, respectively.

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References

[1] C. Toyoshima: Biochim. Biophys. Acta **1793** (2009) 941.
[2] *Y. Norimatsu, K. Hasegawa, N. Shimizu and C. Toyoshima: Nature* ***545*** *(2017) 193.*
[3] J.A. Killian and G. von Heijne: Trends Biochem. Sci. **25** (2000) 429.