Crystal Structure of the Sodium-Potassium Pump and Its Regulation by Cardiac Glycosides

Sodium-potassium ATPase is an ATP-powered ion pump that establishes concentration gradients for Na^+ and K^+ across the plasma membrane in all animal cells by pumping Na^+ from the cytoplasm and K^+ from the extracellular medium. Such gradients are used for many essential processes, notably for generating action potentials. Na^+,K^+-ATPase is a member of the P-type ATPases that include sarcoplasmic reticulum Ca^{2+}-ATPase, the crystal structures of which have been determined for 9 different intermediates that cover nearly the entire reaction cycle [1]. Crystallography of Na^+, K^+-ATPase is far more difficult, partly because heavily glycosylated β-subunit and a regulatory FXYD protein are integral parts of this ATPase in addition to the catalytic α-subunit. We published the first high resolution crystal structure of this important ion pump from shark rectal gland fixed in a state analogous to E2•2K^+•Pi (Fig. 1), in which the ATPase has a high affinity to K^+ and still binds Pi [2]. Clearly visualised at 2.4 Å resolution were co-ordination of K^+ and associated water molecules in the transmembrane binding sites and a phosphate analogue (MgF_4^{2-}) in the phosphorylation site. The crystal structure showed that the β-subunit plays a critical role in K^+ binding, by stabilising the kinked part of a transmembrane helix M7 (Fig. 2). This kink is caused by another kink in M5, originating from a Pro that effectively makes a larger space than Gly, the corresponding residue in Ca^{2+}-ATPase, for accommodating K^+. The involvement of the β-subunit explains, at least partially, why the homologous Ca^{2+}-ATPase countertransports H^+ rather than K^+, although the co-ordinating residues are almost identical.

Cardiac glycosides, prescribed for congestive heart failure for more than two centuries, are efficient inhibitors of Na^+,K^+-ATPase. Their therapeutic index is, however, low and structural details on their binding have been keenly sought. In the subsequent paper [3], we reported a 2.8 Å resolution crystal structure of this ATPase with bound ouabain (Fig. 3(a)), a representative cardiac glycoside derived from a plant *Strophanthus gratus*, introduced by soaking the E2•2K^+•MgF_4^{2-} crystals. Ouabain was deeply inserted into the transmembrane domain with the lactone ring very close to the bound K^+, in marked contrast to previous models (Fig. 3). Due to antagonism between ouabain and K^+, the structure represents a low-affinity ouabain bound state. Yet, most of the mutagenesis data obtained with the high-affinity state are readily explained by the present crystal structure, indicating that the binding site for ouabain is essentially the same and a high affinity is conferred by closure of the binding cavity. All the diffraction data were collected at beamline **BL41XU**.

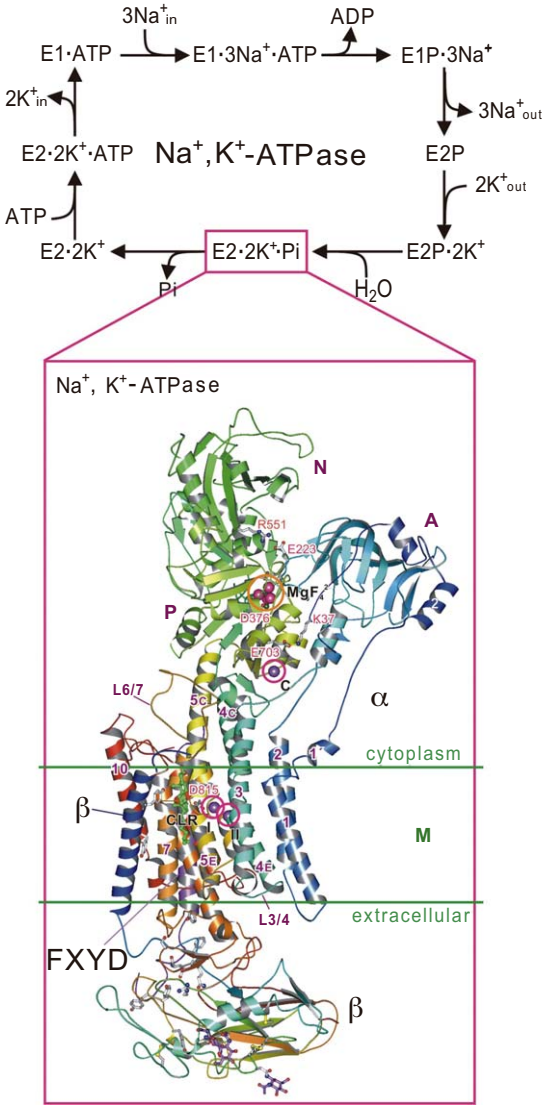

Fig. 1. The reaction cycle and a 2.4 Å resolution crystal structure of Na^+, K^+-ATPase in the E2•2K^+•MgF_4^{2-} form, representing the E2•2K^+•Pi state. According to the classical Albers-Post mechanism, the ATPases have a high affinity in the E1 state and a low affinity in E2 for the ion transferred from the cytoplasm across the membrane (i.e., Na^+); the affinity is reversed for the ion countertransported (K^+). During the reaction cycle, the ATPase is autophosphorylated by ATP at an aspartyl residue. Na^+ bound to the transmembrane binding sites are occluded in E1P, and released to the opposite side of the membrane in the E2P ground state, and the binding sites become occupied by the counterions (K^+). Hydrolysis of the aspartylphosphate and transition into the product state (E2•Pi) closes the extracellular (lumenal) gate and the counterions become occluded. K^+ (purple spheres) and MgF_4^{2-} (orange circle) are shown in space fill.

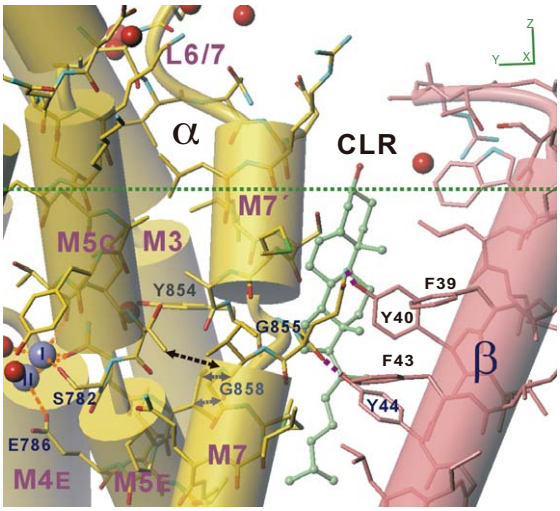

Fig. 2. Details around the unwound part of the M7 helix. Purple spheres represent bound K^+ (I, II). A cholesterol molecule (CLR) is depicted in ball-and-stick. Note that the Gly855 carbonyl is stabilized by a hydrogen bond with Tyr44 of the β-subunit. The horizontal green line shows the approximate boundary of the hydrophobic core of the lipid bilayer.

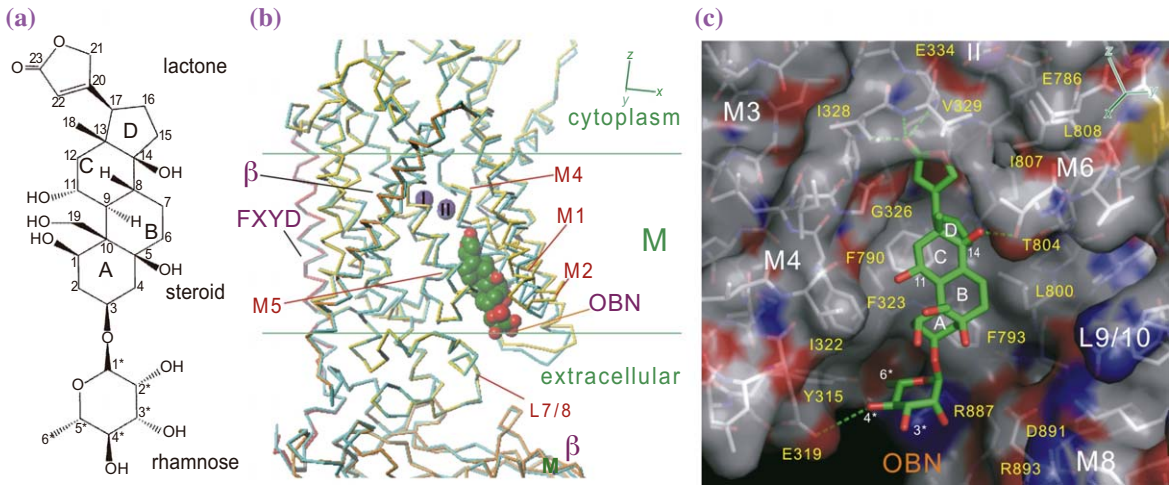

Fig. 3. Crystal structure of Na^+,K^+-ATPase with bound ouabain. **(a)** A diagram of ouabain (OBN). **(b)** Superimposition of Cα traces of Na^+, K^+-ATPase in ouabain bound (yellow) and ouabain unbound (cyan) forms, viewed in the same direction as in Fig. 1. Ouabain (green and red) and K^+ ions (I, II; purple) are shown in space fill. Green horizontal lines indicate the approximate position of lipid bilayer. **(c)** Details of the ouabain binding site viewed parallel to the membrane. Water accessible surface in atom color is superimposed on the atomic model.

Chikashi Toyoshima* and Haruo Ogawa

Institute of Molecular and Cellular Biosciences,
The University of Tokyo

*E-mail: ct@iam.u-tokyo.ac.jp

References

[1] *C. Toyoshima: Biochim. Biophys. Acta* ***1793*** *(2009) 941.*
[2] *T. Shinoda, H. Ogawa, F. Cornelius and C. Toyoshima: Nature* ***459*** *(2009) 446.*
[3] *H. Ogawa, T. Shinoda, F. Cornelius and C. Toyoshima: Proc. Natl. Acad. Sci. USA* ***106*** *(2009) 13742.*