

Crystal structure of Na⁺,K⁺-ATPase with bound sodium ion

Na⁺,K⁺-ATPase is one of the representative members of P-type ATPase family that includes sarcoplasmic reticulum Ca²⁺-ATPase (SERCA) and gastric H⁺,K⁺-ATPase. Na⁺,K⁺-ATPase transports three sodium ions (Na⁺) out and two potassium ions (K⁺) into the cell per molecule of ATP hydrolyzed (Fig. 1). It is expressed in all animal cells and is implicated in many fundamental processes in life, notably excitation of nerve cells and contractions of heart muscle. In the reaction cycle, there are two

major states, E1 and E2 [1,2]. E1 is a state with a high affinity for Na⁺ and E2 has a low affinity for Na⁺ but a high affinity for K⁺. It has been an interesting question how Na⁺,K⁺-ATPase selectively and efficiently transports ions into or out the cells. In 2009, our group determined a crystal structure of Na⁺,K⁺-ATPase in E2·P_i with two bound K⁺ ions at 2.4 Å resolution and showed how this enzyme selectively binds K⁺ [3]. Furthermore, we recently succeeded in determining the crystal structures of this ATPase in E1~P·ADP with three bound Na⁺ at 2.8 Å resolution using **BL41XU** [4].

Na⁺,K⁺-ATPase consists of three components: α-subunit (the catalytic unit), a heavily glycosylated β-subunit, and a tissue-specific auxiliary regulatory polypeptide known as FXYD proteins (Fig. 1). α-subunit has four distinct domains: cytoplasmic actuator (A), phosphorylation (P) and nucleotide binding (N) domains and a transmembrane domain. The crystal structures show three Na⁺ ions bound in the transmembrane (M) domain and provide two major conclusions for selective transport of ions by Na⁺,K⁺-ATPase. One is that this enzyme binds three Na⁺ ions sequentially and cooperatively. Na⁺ binding to the first site (site III), the unique site to Na⁺,K⁺-ATPase, leads to a formation of the second binding site (site I). Then, Na⁺ binding to site I creates the third binding site (site II) by repositioning of the extracellular half of the transmembrane helix of M4 (M4E). Such sequentially and cooperatively formed Na⁺ binding sites, especially site III, are highly constrained to fit only Na⁺ (ionic radius = 0.95 Å) and not K⁺ (1.33 Å) (Fig. 2). In addition, the short distance (3.4 Å) between Na⁺ ions bound at sites I and II indicates that larger K⁺ or divalent cations (e.g. Ca²⁺) cannot bind because of the physical size and/or electrostatic repulsion.

Another major conclusion is that only the binding of the right-sized ion (i.e. Na⁺) to site III allows productive phosphoryl transfer from ATP. Site III is located in the hinge between the two halves of the M5 helix (M5C and M5E) and controls the position of the P-domain, as M5C is integrated into the P-domain. For phosphoryl transfer to take place, a proper arrangement of the three cytoplasmic domains is necessary. Such an arrangement is realized only by the binding of the right-sized ion to site III.

The crystal structure also shows how oligomycin, an antibiotic, blocks the activity of Na⁺,K⁺-ATPase. Oligomycin is known to bind to Na⁺,K⁺-ATPase in E1P but not in E2P, unlike cardiotonic glycosides such as ouabain and digitoxin. In the structure complexed

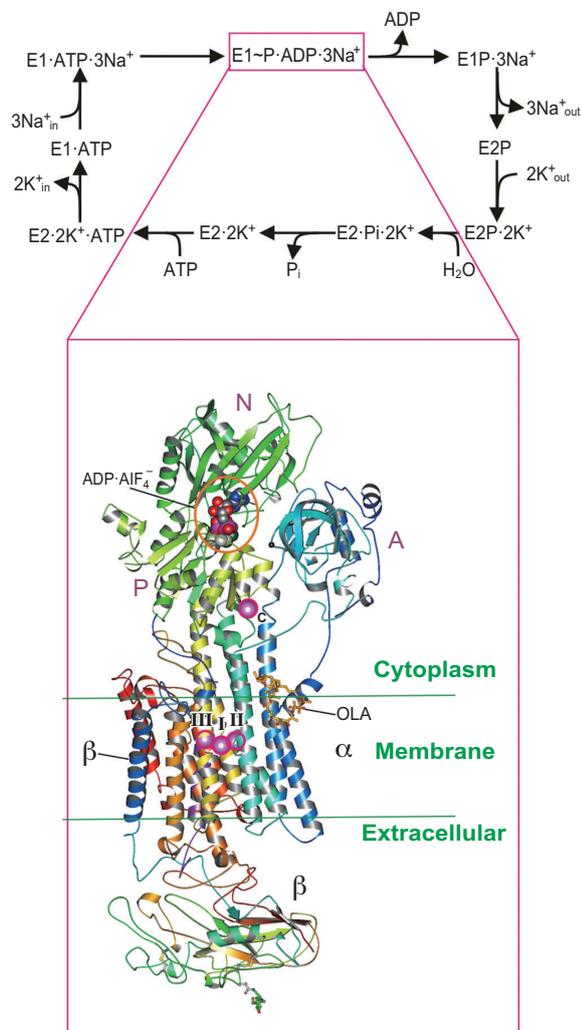


Fig. 1. The reaction cycle and a crystal structure of Na⁺,K⁺-ATPase in E1~P·ADP·3Na⁺. Top panel shows a simplified reaction cycle. Bottom panel shows the whole complex. Na⁺,K⁺-ATPase consists of α and β subunits and an auxiliary FXYD protein. The α-subunit is the catalytic subunit, consisting of three cytoplasmic domains and 10 transmembrane helices. Violet spheres represent bound Na⁺ (I-III and C) and OLA is oligomycin.

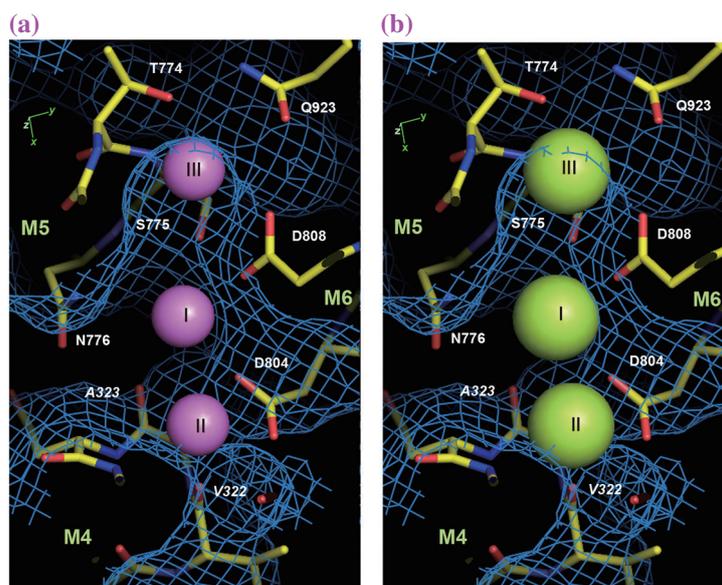


Fig. 2. Continuous cavity connecting three Na⁺-binding sites. Na⁺ accessible surface (blue net) with three Na⁺ (a, violet spheres) and K⁺ (b, green spheres) placed in the Na⁺ binding sites. In (b) it is evident that the cavity is too small for K⁺.

with oligomycin, it is located at the junction of M1 and M1' and sterically blocks the transition into the E2P state (Fig. 3).

Na⁺,K⁺-ATPase is the target of digitalis, a cardiotonic glycoside prescribed for more than two

centuries, and is implicated in many diseases, such as high blood pressure, neurological disorders and cancers. The crystal structure described here provides a solid basis for developing drugs that regulate the activity of Na⁺,K⁺-ATPase.

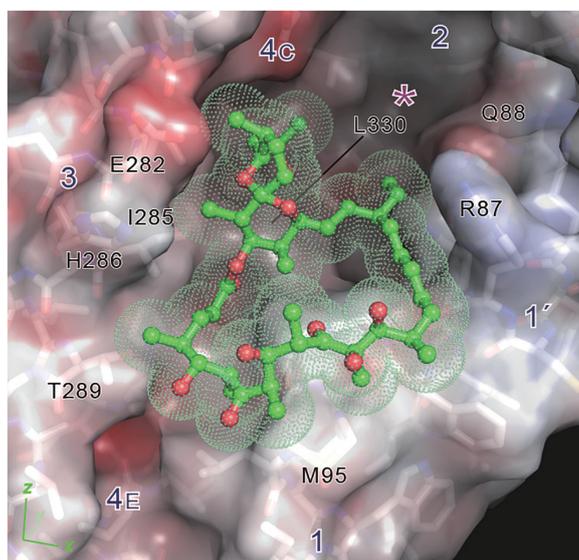


Fig. 3. Binding cavity for oligomycin; surface representation, colored according to the surface potential (blue, positive; red, negative). Green dotted spheres show van der Waals surface of oligomycin.

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