Crystal structures of the gastric proton pump reveal the mechanism for proton extrusion

After intaking food, the pH inside our stomach reaches around 1. This acidic environment, generated by the gastric proton pump H^+,K^+-ATPase [1], is indispensable for food digestion and is also an important barrier to pathogen invasion via the oral route. However, excess stomach acidification induces ulcers, which considerably impair the health of those affected. Acid suppression in combination with antibiotics is a widely recognized treatment to eradicate *Helicobacter pylori*, a strong risk factor for gastric cancer. Proton pump inhibitors (PPIs) and a recently developed class of acid suppressants called K^+-competitive acid blockers (P-CABs) are commonly used for the treatment of acid-related diseases. Gastric H^+,K^+-ATPase therefore continues to be a prominent target for the treatment of excess stomach acidification.

Similarly to other P-type ATPases, gastric H^+,K^+-ATPase mediates the uphill transport of H^+ and K^+ fueled by ATP hydrolysis (Fig. 1), which is accomplished by the cyclical conformation changes of the enzyme. H^+,K^+-ATPase is composed of two subunits. The catalytic α-subunit is highly homologous to those of related P2-type ATPases such as Na^+,K^+-ATPase and sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA). In addition, H^+,K^+-ATPase requires an accessory β-subunit for functional expression as an αβ-complex.

We determined the crystal structures of gastric H^+,K^+-ATPase in a luminal-open E2P conformation bound to vonoprazan or SCH28080, representative P-CABs, which were analyzed to 2.8 Å resolution at SPring-8 **BL32XU** and **BL41XU** [2]. The α-subunit comprises 10 transmembrane helices (M1-M10), in which a cation-binding site is located, and three cytoplasmic domains – the nucleotide (N), phosphorylation (P), and actuator (A) domains (Fig. 2(a)). The β-subunit has a single TM helix and a large ectodomain with three of the six *N*-linked glycosylation sites visualized in the structure. The electron density maps define the binding mode of vonoprazan (now available for clinical treatment) and SCH28080 (a prototype of P-CAB), and the residues coordinating them, in a luminal-facing conduit that extends to the cation-binding site (Figs. 2(b) and 2(c)). The binding sites of these P-CABs were previously thought to overlap owing to similar inhibitory actions. Our structures show that they do indeed partially overlap but are also distinct. The binding mode of P-CABs determined in the crystal structure is consistent with mutagenesis studies, providing the molecular basis for P-CAB binding to H^+,K^+-ATPase, which will be useful for the structure-based development of novel PPIs.

According to the transport scheme, the luminal-open E2P state is an intermediate state occurring just after proton release and allows subsequent K^+ binding. In fact, the cation-binding site is exposed to the luminal bulk medium when bound P-CAB is removed from our structures. Similarly to other P2-type ATPases

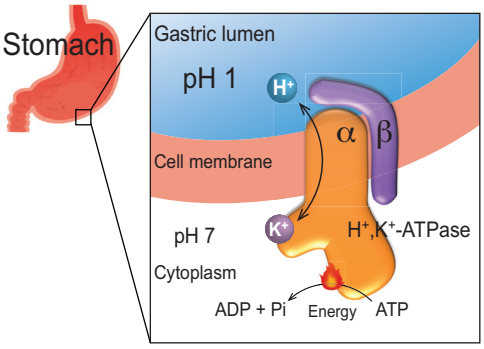

Fig. 1. Gastric proton pump H^+,K^+-ATPase.

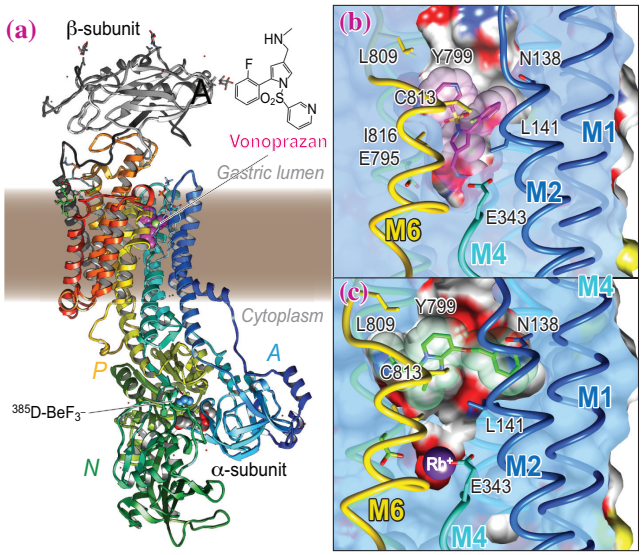

Fig. 2. Overall structure of gastric H^+,K^+-ATPase **(a)**. The color of the α-subunit gradually changes from the N terminus (blue) to the C terminus (red), and is gray for the β-subunit. Vonoprazan bound to the transmembrane region is shown as a magenta sphere. The sectional surfaces of vonoprazan- **(b)** and SCH28080- **(c)** bound structures are shown. Several amino acids important for P-CAB binding are indicated in the figure.

and cation-transporting proteins, H^+,K^+-ATPase also utilizes conserved acidic amino acids of its cation-binding site in M4 and M6 for H^+ transport, except for a lysine residue in M5. This invariant lysine (Lys791) in H^+,K^+-ATPase is replaced with serine in Na^+,K^+-ATPase, and is thus predicted to be important for proton transport. In our structure, the carboxyl residue of Glu820 is surrounded by other polar amino acids (Figs. 3(a) and 3(b)). The juxtaposition of the two glutamates (Glu795 and Glu820, 2.5 Å between their closest oxygens) indicates that at least one of these acidic residues is protonated. Because the charge-neutralized Glu795Gln mutant shows an ATPase activity profile comparable to the wild-type enzyme, in this case, Glu795 is likely to be protonated. Therefore, these two glutamate residues interact through a hydrogen bond. Glu820 also receives hydrogen bonds from Asn792 (3.0 Å) and a water molecule (3.5 Å). In addition to this hydrogen bond network around Glu820, the ε-amino group of Lys791 interacts intimately with the carboxylate of Glu820 (3.1 Å). Thus, the Glu820 carboxyl is situated in an unusual environment with extensive polar interactions that could lower its pK_a value. A reduction of pK_a values in juxtaposed carboxyl groups of two adjacent acidic residues occurs in the catalytic centers of many other enzymes; for example, two aspartate residues 2.5 Å apart in the catalytic center of pepsin were estimated to have pK_a values of 1.2 and 4.7 [3]. Therefore, H^+,K^+-ATPase Glu820 is a strong candidate for the proton release site, which enables the generation of the pH 1 solution in the stomach.

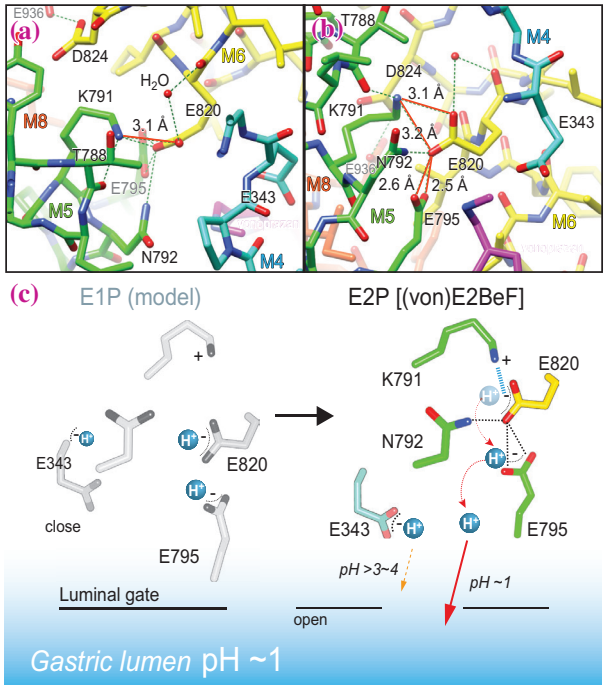

Fig. 3. Close-up of the cation-binding site in H^+, K^+-ATPase, viewed approximately perpendicular to the membrane from the cytoplasmic side **(a)** and parallel to the membrane from the M4 side **(b)**. Dotted lines are drawn between residues within 3.5 Å of neighboring atoms. The interactions between Glu795 and Glu820 (hydrogen bonds) and between Lys791 and Glu820 (salt bridges) are highlighted as red lines with the distances between them indicated. **(c)** Model for proton extrusion into the acidic solution. In the H^+-occluded E1P state (left), all three glutamates in the cation-binding site are protonated; otherwise, H^+ would be incorporated into the cation-binding site owing to its high concentration in the stomach when the luminal gate opens. In the luminal-open E2P state (right, based on the present structure), the H^+-binding affinity of the Glu820 carboxyl is greatly reduced because of its juxtaposition with Glu795, a hydrogen bond to Asn792, and a salt bridge with Lys791. As a consequence, a single H^+ is expelled into the luminal acidic solution.

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