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## A model of 3D-structure of H<sup>+</sup>, K<sup>+</sup>-ATPase catalytic subunit derived by homology modeling

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### ABSTRACT

**AIM:** To build a model of 3D-structure of H<sup>+</sup>, K<sup>+</sup>-ATPase catalytic subunit for theoretical study and anti-ulcer drug design. **METHODS:** The model was built on the basis of structural data from the Ca<sup>2+</sup>-ATPase. Structurally conserved regions were defined by amino acid sequence comparisons, optimum interconnecting loops were selected from the protein databank, and amino (N)- and carboxyl (C)-terminal ends were generated as random coil structures. Applying molecular mechanics method then minimized the model energy. Molecular dynamics technique was used to do further structural optimization. **RESULTS:** The model of 3D-structure of H<sup>+</sup>, K<sup>+</sup>-ATPase was derived. The model is reasonable according to several validation criteria. There were ten transmembrane helices (TM1-TM10) in the model and inhibitor-binding site was identified on the TM5-8 riched negatively charged residues. **CONCLUSION:** The 3D-structure model from our study is informative to guide future molecular biology study about H<sup>+</sup>, K<sup>+</sup>-ATPase and drug design based on database searching.

### INTRODUCTION

H<sup>+</sup>, K<sup>+</sup>-ATPase is a member of the P-type ATPase that responsible for acid secretion into the stomach and catalyzes electro neutral exchange of cytoplasmic H<sup>+</sup> and external K<sup>+</sup> coupled with ATP hydrolysis<sup>[1]</sup>. It is present in gastric parietal cells of various higher animals such as human, rabbits, pigs, dogs, rats, and frogs and is located in cytoplasmic vesicles or apical plasma membranes of the secretary canaliculus. H<sup>+</sup>, K<sup>+</sup>-ATPase is composed of two subunits, the alpha subunit, which contains the phosphorylation site, is the catalytic subunit, and the beta subunit, which has six or seven

N-linked glycosylation sites. It has been shown that the H<sup>+</sup>, K<sup>+</sup>-ATPase inhibitors bind to transmembrane helices of the alpha subunit, but the function of beta subunit is not so clear<sup>[2-4]</sup>. An accurate, high-resolution picture of the inhibitor-binding site will await determination of the 3-D structure of the alpha subunit of a H<sup>+</sup>, K<sup>+</sup>-ATPase.

The alpha subunit of human H<sup>+</sup>, K<sup>+</sup>-ATPase is comprised of about 1035 amino acid residues ( $M_r=114\ 047$ )<sup>[5]</sup>, these residues are thought to be arranged in 10 transmembrane (TM) segment. The evidences have been obtained through biochemistry and molecule biology studies that covalent inhibitors, “razoles (such as omeprazole, pantoprazole, *etc*)”, react with cys<sup>813</sup> in the intercellular M5-M6 region of the H<sup>+</sup>, K<sup>+</sup>-ATPase. Trypsinolysis followed by tricine gradient gel separation of the SDS-solubilized membrane fraction and sequencing provided details of the sequences of the first eight membrane segments, as did labeling either with

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thiophilic reagents such as omeprazole, lansoprazole or pantoprazole (M3/4, M5/6, and M7/8) or with imidazopyridine derivatives of SCH28080<sup>[6,7]</sup>. A combination of tryptic cleavage, peptide sequencing and *in vitro* translation tests demonstrated that covalent ligands bind to a common site, Cys 813 in external surfaces.

Knowledge of the three-dimensional structure can be great importance for the drug design. Structural information often greatly enhances our understanding of proteins function. X-ray crystallography, electron microscopy or diffraction and NMR spectroscopy are the three techniques to obtain actual 3D-structure of proteins. However, H<sup>+</sup>, K<sup>+</sup>-ATPase has not yet been crystallized, and the main disadvantage of NMR for solving H<sup>+</sup>, K<sup>+</sup>-ATPase is that high concentration of dissolved H<sup>+</sup>, K<sup>+</sup>-ATPase is needed, but H<sup>+</sup>, K<sup>+</sup>-ATPase refuse to dissolve in normal solvents. In the absence of experimental data, model building on the basis of the known three-dimensional structure of a homologous protein is at present the only reliable method to obtain structural information. In this article, we built the 3D-structural model of H<sup>+</sup>, K<sup>+</sup>-ATPase based on the known 3D-structure of Ca<sup>2+</sup>-ATPase<sup>[8]</sup> with homology method.

## COMPUTATIONAL METHODS

Model building by homology is a multistep process. The process proposed by the followed steps: template recognition, sequence alignment, backbone and canonical loops generation, side chain generation plus optimization, and overall model optimization.

**Template recognition and amino acids sequence alignment** A BLASTP search using the Human Gastric H<sup>+</sup>, K<sup>+</sup>-ATPase alpha chain sequence (ATHA, swisspot ID: P20648) as the query sequence matched only one template, 1EUL, the crystal structure of Ca<sup>2+</sup>-ATPase at 2.6 Å resolution. After finding the template, a pair wise sequence alignment between 1EUL and ATHA was obtained by ClustalW program. Then, removing the m-boxes and using the Modeler/Align2D command in Homology performed alignment correction.

**3D model building** Backbone of the protein's core regions were transferred directly from the corresponding coordinates of 1EUL. Side chains conformation for backbone residues was generated automatically by Homology. Coordinates for the remaining residues in the loops were obtained from PDB database using a fragment-searching approach. The 10 best candidate structures of the loops was extracted from PDB database according to the minimum RMS deviation of the

C $\alpha$  trace between the residues adjacent to the fragment considered (5 adjacent residues per sides). The lowest RMS loop was then selected on the basis of the analysis result of the Profile-3D program that measured the compatibility between the protein sequence profile and its 3D profile. The (C)- and (N)- terminals of the model were generated automatically through end-repair with Homology. The resulting model was subjected to an energy minimization by using the Refine routine in Homology. Using the parameters as a distance-dependent dielectric constant  $\epsilon=1.0$  and non-bonding cutoff of 15 Å, AMBER force field and Amber-all-atom charges, a 800-step steepest descent minimization was first performed until the maximum derivative is less than 50.0 kcal·mol<sup>-1</sup>·nm<sup>-1</sup>, and then a 1000-iteration conjugate gradient minimization was performed until the maximum derivative is less than 20.0 kcal·mol<sup>-1</sup>·nm<sup>-1</sup>. The above energy minimizations were started with the core main chain, then all the core side chains, the loop main chain, and finally all the loop side chain. The whole model was minimized to convergene.

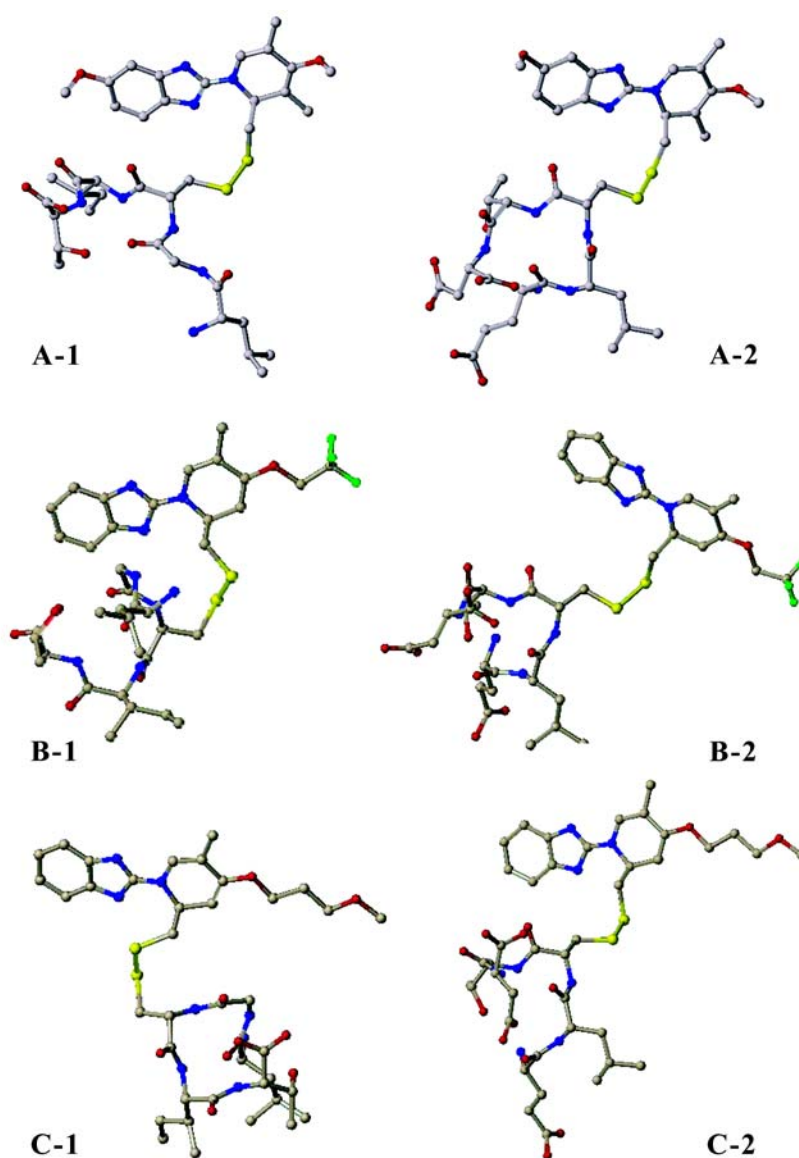
In the next step, the model was subjected to molecular dynamics (MD) simulations using the same parameters and geometrical constrains as described above for 19 000 iterations. The model was kept 300 K during the entire simulation and wrote history file every 1000 steps. The averaged structure from the last 20 ps of the MD was then calculated and further optimized with steepest descent and conjugate gradient methods to generate the final refined structural model of H<sup>+</sup>,K<sup>+</sup>-ATPase.

## RESULTS AND DISCUSSION

**Amino acid sequence alignment and homology modeling** Structural alignment of ATHA with the template ATA1\_RABIT was shown in Fig 1. The sequences are highly conserved for the helices M4-M6 and M8 (shaded in Fig 1). This alignment was chosen not only according to the aligning score but also based on the natural sequence variability. This is because lipid-facing residues tend to be variable, whereas residues involved in helix-helix contacts tend to be conserved. Firstly, the structural checking was performed using the ProtStat in the Insight II/Homology. Some structural flaws produced by incorrectly packed side chains, distortions in incorrectly aligned regions, were found and corrected till all the bond lengths and dihedrals were in the normal range of geometrical criteria. And then, the Profiles-3D in Homology module was applied to





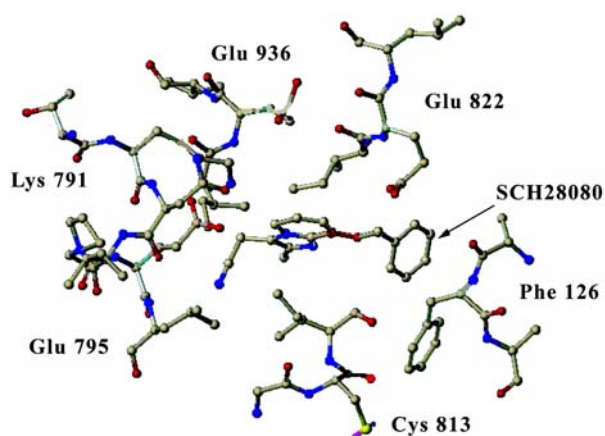


**Fig 4.** The razole-cysteine complexes (hydrogen were not present). A-1: omeprazole with Cys813; A-2: omeprazole with Cys822; B-1: lansoprazole with Cys813; B-2: lansoprazole with Cys822; C-1: rabeprazole with Cys813; C-2: rabeprazole with Cys822.

a favorable energy of SCH28080 and enzyme complex was obtained. In this complex, phenyl ring of the Phe<sup>126</sup> toward to the extracellular surface of the enzyme, the conformation of the SCH28080 like a bow integrates in the pocket surround by Glu<sup>795</sup>, Glu<sup>822</sup>, Glu<sup>936</sup>, Phe<sup>126</sup> and Cys<sup>813</sup>. The phenyl of SCH28080 interacted with the phenyl of Phe<sup>126</sup>, and the polar part interacted with negatively charged residues (Fig 5).

This work offered a computed model of gastric H<sup>+</sup>, K<sup>+</sup>-ATPase catalytic subunit, it provided a valuable insight into the structural of the enzyme and the structural environments of the inhibitors binding sites. Struc-

tural information of the H<sup>+</sup>, K<sup>+</sup>-ATPase is lacking, but the homology modeling method provided a model suggesting a mechanism for inhibitors interacting with the enzyme through comparing theoretical 3D-structural data with the biochemical information of P-type ATPase. The model proposes that the electrostatic and hydrophobic factors around the binding sites influence the inhibitors interacting with the enzyme, and the reversible inhibitor SCH28080 partially spans the extracellular surface of the enzyme to interact with residues in the M1 to M6 transmembrane domains. These information could provided some useful information for new



**Fig 5.** SCH28080 binding with the active pocket (hydrogen atoms were not present).

class of inhibitors, but the more detail information was waiting for the farther structural information becoming available.

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