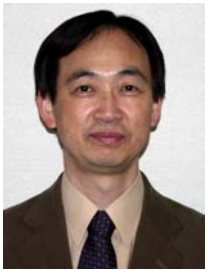
【Grant-in-Aid for Specially Promoted Research】
Biological Sciences



Title of Project：Structural biology of membrane transporters with a view to drug development

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Research Area：Biology

Keyword：ion pumps, membrane proteins, crystallography, energy conversion

【Purpose and Background of the Research】

The project has two aims: one is the complete structural understanding of active transport, which requires comparison of different pumps in different states as well as mutants. The other is structure determination of transporters of infectious organisms for facilitating drug development.

We have determined crystal structures of Ca^{2+}-ATPase in 9 different states and those of Na^+,K^+-ATPase in 2 states, which enabled us to describe the scenario of ion pumping in fair detail. Nevertheless, our understanding on "why the structure has to be so" or "how the free energy provided by ATP hydrolysis is utilised" still remains to be primitive. We approach these fundamental questions in this project.

During structure determination, we also acquired knowledge on inhibitors of pumps. Pumps may be particularly good targets for killing infectious organisms. It also appears quite possible to carry out good structural studies with pumps and transporters of, for instance, malaria parasites or *Mycobacterium tuberculosis*, and thereby contribute to human welfare at the same time.

【Research Methods】

To address these questions we need to determine mutant structures, carry out molecular dynamics simulations and measure thermodynamic parameters for every partial reaction. These include X-ray crystallography of recombinant membrane proteins. For that purpose, we have established a system for large-scale production using adenovirus-COS1 cells.

【Expected Research Achievements and Scientific Significance】

a. Structural study on Ca^{2+}-ATPase and its mutants: Here we are going to determine the crystal structure of the ATPase in the E1 state, as it will tell us what is the activation signal for phosphoryl transfer elicited by the binding of Ca^{2+}, and that of the mutants of Glu309, which is the gating residue of Ca^{2+}-binding sites, to reveal the structural changes achieved by the first Ca^{2+} binding. These crystal structures will nearly complete the structural account of ion pumping.

The atomic structures of the ATPase will be correlated to thermodynamic measurements on partial reactions to understand how the energy of ATP is utilised, which is one of the most fundamental questions in modern biology.

b. Structural study on Na^+,K^+-ATPase and its complexes with drugs and proteins: Other than crystal structure determinations of the ATPase in the E2K and E1~P states, we plan to study the complexes of the ATPase with cardiotonic steroids of therapeutic vales and those with various proteins, e.g. Src kinase and the IP3 receptor, which form large signal complexes.

c. Structural determinations of malarial P-type ATPases: The impacts gained by crystal structures of pumps and transporters involved in infectious diseases will require no explanation, as it will greatly facilitate development of efficient drugs.

【Publications Relevant to the Project】

T. Shinoda, H. Ogawa, F. Cornelius and C. Toyoshima: Crystal structure of the sodium-potassium pump at 2.4 Å resolution. *Nature* **459**, 446-450 (2009)

C. Toyoshima, Y. Norimatsu, S. Iwasawa, T. Tsuda and H. Ogawa: How processing of aspartylphosphate is coupled to lumenal gating of the ion pathway in the calcium pump. *Proc. Nat. Acad. Sci. USA.* **104**, 19831-19836 (2007)

【Term of Project】 FY2011-2015

【Budget Allocation】 399, 600 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.iam.u-tokyo.ac.jp/StrBiol/index.html