X-ray Crystallography of Ultra-thin Membrane Protein Crystals

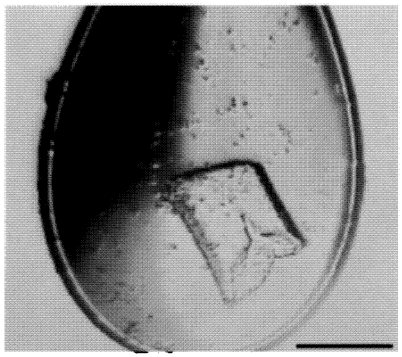
Chikashi Toyoshima (3795)*, Haruo Ogawa (3796) and Hiromi Nomura (4635)
Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan.

The purpose of this project is to establish the methods required for crystallography of ultra-thin protein crystals often found with membrane proteins. A typical example is Ca^{2+}-ATPase from muscle sarcoplasmic reticulum (SR), a representative member of P-type ATPases, which are involved in active ion transport across the membrane. SR Ca^{2+}-ATPase forms very thin plate-like crystals when high concentration of Ca^{2+} is present. We have succeeded in determining the structure to 2.6 Å resolution with two Ca^{2+} bound in the transmembrane region (*Nature* 405: 647-655, 2000) and are now trying to visualise the conformation changes implicated in the active transport.

Mg/F complex of Ca^{2+}-ATPase has been regarded as a stable analogue of the phosphorylated form (so-called 'E2P' form) after transferring 2 Ca^{2+} into the lumenal side. This complex is formed in the absence of calcium and very stable. As described in our previous report (2000B0263-CL –np), we can now grow crystals of this form very reproducibly by dialysis method (Figure).

We also reported that these crystals are however very sensitive to changes in temperature and other conditions. Therefore, we devised a simple apparatus for freezing in a cold room. It is a kind of guillotine consisting of a stainless Dewer flask and silicon rubber heater for making cold nitrogen gas. This apparatus allows freezing of many crystals in a very short time at a faster cooling rate, because the flow of cold gas can be easily adjusted.

The crystals thus prepared diffracted to 3.0 Å resolution at BL41XU. They belonged to *C2* space group and had the unit cell dimensions of a = 176.4, b = 70.1, c = 142.6 Å and β = 106.9°. Structure determination by molecular replacement was successful and revealed very large domain movements. Interestingly the structure around the phosphorylation site was virtually identical to other members of haloacid dehalogenase superfamily, namely, phosphoserine phosphatase (with PO_4 or BeF_3) and with phosphonatase (with WO_4), atomic co-ordinates of which were published recently. In particular, the atomic model of phosphoserine phosphatase was directly superimposable with the electron density map of Ca^{2+}-ATPase, even in the associated helices of the central Rossmann fold. This similarity actually extends even further to bacterial chemotaxis regulator proteins, which have different folds but share the same amino acid residues around the phosphorylation sites.



Crystals of Mg^{2+}/F^- complex of sarcoplasmic reticulum Ca^{2+}-ATPase. The bar represents 200 μm.

Crystal structure analysis of γ-glutamylcysteine synthetase from *Escherichia coli* B

***Takao Hibi[1] (5092), Tomohiro Nago[1] (5563), Yoichi Kurokawa[1] (6640), Toru Nakatsu[2] (1611), Hiroaki Kato[2] (1610), Jyu'ichi Oda[1] (5093)**
[1]Department of Bioscience, Fukui Prefectural University, Kenjyoujima, Fukui, Japan 910-1195, [2]Harima Institute, RIKEN, Koto, Hyogo, Japan 679-5148

γ-Glutamylcysteine synthetase (γGCS), which catalyzes the ATP-dependent coupling of L-Glu and L-Cys to form a glutathione precursor γ-L-Glu-Cys, is the rate-limiting enzyme in glutathione biosynthesis. The γGCS is one of targets of potential therapeutic agents such as a parasiticide or a drug suppressing multi-drug resistance of cancer cells. Here we report the structure analysis of γGCS from *E. coli* B on the MAD method.

The Se-Met mutant of γGCS was obtained by the pathway inhibition method. Using the sitting-drop vapor-diffusion technique, the Se-Met derivative crystals grew to maximum dimensions of 0.3 × 0.25 × 0.2 mm (SeMetGCS1) and 0.2 × 0.15 × 0.15 mm (SeMetGCS2) within a month. Both of the derivative crystals were dialyzed against the mother liquor containing 20% glucose for flash cooling. In the case of SeMetGCS2 crystal, 1mM cysteine was added into the liquor.

The XAFS spectrum of Se-Met derivative showed a clear peak and edge. MAD data sets of Se-Met derivative crystals were collected at 100 K using a MAR CCD detector and synchrotron radiation on beam line BL41XU. The exposure time and oscillation angle per flame were 30 sec and 1.0° (total oscillation range, 180°). Intensity data were processed using MOSFLM and the CCP4 program. Processing data revealed a hexagonal crystal system *R*3 with unit-cell parameters a = b = 327.0 Å, c = 104.5 Å. Data collection and processing statistics are shown in Table 1. Further data analysis is now underway.

Table 1: Summary of data collection

SeMetGCS1	edge	peak	remote
energy (keV)	12.6587	12.6612	12.6015
camera distance (mm)	170	170	170
resolution (Å)	2.8	2.8	2.8
total reflections	577,902	586,705	592,176
unique reflections	102,101	102,110	102,168
R_{merge} (%)	6.2 (23.9)	5.8 (18.4)	7.6 (31.0)
I/σ	10.4 (3.0)	10.5 (3.9)	8.6 (2.3)
Completeness (%)	99.6	99.8	99.9
Ave. redundancy	5.6	5.7	5.8
SeMetGCS2	**edge**	**peak**	**remote**
energy (keV)	12.6587	12.6612	12.7260
camera distance (mm)	185	185	185
resolution (Å)	3.0	3.0	3.0
observed reflections	455,396	467,345	468,428
unique reflections	83,330	83,316	83,900
R_{merge} (%)	7.7 (25.4)	7.4 (24.1)	9.4 (41.0)
I/σ	8.8 (2.9)	9.0 (3.0)	7.4 (1.8)
Completeness (%)	98.9	99.3	99.2
Ave. redundancy	5.5	5.6	5.6