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X-ray Crystallography of the Calcium Pump of Sarcoplasmic Reticulum

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The purpose of this project is to studies the structures of Ca^{2+}-ATPase from muscle sarcoplasmic reticulum (SR) in various physiological states. SR Ca^{2+}-ATPase is a representative member of P-type ATPases that are responsible for establishing ion gradients across biological membranes by transporting ions against concentration gradients using chemical energy of ATP. We have already published the structure of this ion pump with two Ca^{2+} bound in the transmembrane region ($E1{\cdot}2Ca^{2+}$ form; *Nature* 405: 647-655, 2000) to 2.6 Å resolution and that in the absence of Ca^{2+} but the presence of thapsigargin, a very potent inhibitor from plant (E2(TG) form, *Nature* 418: 605-611, 2002) to 3.1 Å resolution. Now the $E1{\cdot}2Ca^{2+}$ form is re-refined at 2.4 Å resolution and deposited in the PDB as 1SU4. In addition to these, we have succeeded in making crystals of E1·AMPPCP (with bound Ca^{2+}, AMPPCP and Mg^{2+}; AMPPCP as a nonhydrolysable analogue of ATP), $E1{\cdot}AlF_x$ (with bound Ca^{2+}, AlF_x and Mg^{2+}; AlF_x as a stable phosphate analogue), and $E2{\cdot}MgF_4^{2-}$ (without Ca^{2+}; MgF_4^{2-} as a stable analogue of phosphate) and their structure determinations. Thus, we now have atomic models for all 4 principal states. Of these, we have completed the refinement with the E1·AMPPCP form at 2.9 Å resolution and deposited in the PDB (accession code: 1VFP).

. In addition to these, we are also interested in inhibitors of the Ca^{2+}-ATPase. TG, the most efficient inhibitor (sub-nanomolar affinity), is a sesquiterpene lactone from plant. This is a rather complicated molecule having long hydrophobic tails and occupies the space between the M3 and M7 transmembrane helices, both are not directly involved in Ca^{2+}-binding. It is known that Ca^{2+}-ATPase (SERCA1a) is inhibited by a variety of hydrophobic, hydroxy-containing compounds. These include BHQ (2,5-di-*tert*-butyl-1,4-benzohydroquinone) and curcumin. Biochemical data suggest that all these 3 bind to different binding sites in the E2 state, and work synergistically.

We have succeeded in making crystals with BHQ alone first. It was certain that BHQ was bound to the enzyme, because otherwise Ca^{2+}-ATPase denatures quickly in the E2 condition (*i.e.* in the absence of Ca^{2+}). The crystals grown showed similar morphology to the E2(TG) crystals but smaller. The symmetry and cell dimensions were also very much the same as those of E2(TG), and one cell dimension is very large (nearly 600 Å). Hence, R-Axis V was used with a 90° arc goniometer head. Though the resolution was limited to 4.0 Å, molecular replacement starting from the model built for E2(TG) went out smoothly and yielded a clear Fo-Fc map. The map showed only 3 positive peaks all in the transmembrane region but none at the position occupied by TG in E2(TG).

We have also managed to make crystals in the presence of both BHQ and carcumin. Crystals diffracted somewhat better and yielded a good set of diffraction data to 3.3 Å resolution. However, the peaks found in Fo – Fc map were the same as those with BHQ alone. We would like to collect more data in the next machine time.

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Visualisation of the Lipid Bilayers in the Crystals of Membrane Proteins

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We have been working on the structure determination of Ca^{2+}-ATPase from sarcoplasmic reticulum and have already succeeded in determining its structure in 5 different states at better than 3.2 Å resolution. One interesting aspect of our crystals of Ca^{2+}-ATPase is that they have exogenous lipids, which are expected to form lipid bilayers in the crystals. The presence of very strong lamellar reflections at the positions deviated from the reciprocal lattice points confirmed this idea (Fig.1). Accordingly, these crystals offer a unique opportunity to study the lipid-protein interactions in a great detail.

To visualise the bilayers, we must record very low-resolution reflections that are neglected in the ordinary crystallographic analyses. For phasing of these low resolution reflections, we can utilise MAD information derived from anomalous solvent as the contrast medium. As a contrast medium, we can use aurothioglucose.. Another possibility is to use phospholipids that contain anomalous scatterers. For this purpose, we used brominated phosphatidylcholine (Br-PC) and made crystals. If the ratio of Br-PC to normal PC was not too high, crystals diffracted to a reasonable resolution at BL41XU.

Diffraction data were collected with R-Axis V at a camera length of 600 mm. The anomalous peak was found at λ = 1.0401 Å. In previous runs, a complete set of MAD data consisting of 4 data sets around this wave length were collected from one crystal of SR Ca^{2+}-ATPase soaked in 15% aurothioglucose. However, at this wavelength, the background arising from scattering by air was substantial, and it was necessary to insert beam stop at a short distance from the crystal. Therefore, we could not collect lowest resolution data.

To overcome this problem, a helium path of 500 mm in length with a motor-driven beam stop was constructed and tested in this run. Due to the limitation of time allotted to this experiment, it was impossible to obtain a quantitative measure for the improvement of data quality, but the effect of helium path was obvious. Nevertheless, we could not reduce the size of the beam stop to less than 3 mm square (Fig. 1). This is because the optics setting in front of the specimen is not optimal (having only one single-hole collimator). Thus, at present, it is possible to measure diffraction intensities from ~1/150 $Å^{-1}$ to 1/3.2 $Å^{-1}$.

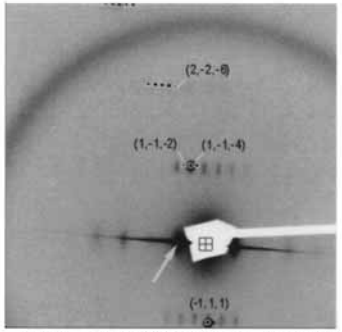

Fig.1 Diffraction pattern of a crystal of Ca^{2+}-ATPase ($P4_1$; $a = b = 71.7$, $c = 590.3$ Å) around the beam stop set in front of the R-Axis V detector at λ = 1.0401 Å. Note the lamellar reflections arising from lipid bilayers in the crystal. The lowest resolution one (arrow) is at ~1/150$Å^{-1}$. Diffraction spots identified by Denzo are circled. The ring comes from Kapton film of the He-path.