X-Ray crystallographic analysis of alginate lyase, a member of family PL-7, from *Pseudomonas aeruginosa*.

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Pseudomonas aeruginosa produces alginate as a capsule like biofilm responsible for both chronic pulmonary infections and respiratory difficulties in the lungs of patients with cystic fibrosis. The bacterial alginate seems to play a crucial role in the adherence of Pseudomonas aeruginosa to target cells. Alginate lyase depolymerizes alginates, a heteropoly-saccharide consisting of α-L-guluronate and β-D-mannuronate, through a β-elimination reaction. A protein PA1167 produced by *Pseudomonas aeruginosa* is a newly identified alginate lyase with a preference for heteropolymeric regions. Therefore, it is important to clarify the relationship between structure and functions of alginate lyase PA1167, which is responsible for the biofilm-dependent ecosystem in Pseudomonas aeruginosa. In this beam time, we are trying to determine the structure of the alginate lyase PA1167 by MAD method by using Br derivative of the enzyme. Based on its primary structure, PA1167 was identified as a member of polysaccharide lyase family PL-7. There is still no literature about the crystal structure of the protein of PL-7.

The Br derivative of the PA1167 from *Pseudomonas aeruginosa* was purified from *Esherichia coli* cells harboring a plasmid-containing gene of the protein. The PA1167 was purified to homogeneity from the *E. coli* cell extract through 3 steps. The purified protein was crystallized by the hanging drop vapor-diffusion method. The crystallization drop was prepared by mixing 3 μl of 1 mg-protein/ml solution with 3 μl of reservoir solution comprising 1.4 M sodium chloride, 0.1 M potassium sodium phosphate and 0.1 M MES buffer at pH 6.5. Crystals of the PA1167 grew in droplets equilibrated against 1.0 ml of the reservoir solution at 293K for 2weeks. Br was introduced by soaking the crystals against 1 M sodium bromide solution for 30 seconds.

The diffraction data for a crystal of the PA1167 up to 2.5Å were collected at 100K under a nitrogen gas stream with an MAR CCD detector using synchrotron radiation (BL-41XU) at three wavelength of 0.9197Å (Peak), 0.9202 Å (Edge) and 0.9170 Å (Remote), and were processed using a program packages of HKL2000. The space group of the crystal was estimated to be $P2_1$ (Monoclinic), with unit cell dimensions of a = 43.5, b = 71.0, c = 67.7 Å and β = 94.6. These three data sets have about 26,105 independent reflections (98% completeness) with an R_{merg} value of 7-9%. The Bijvoet difference Patterson map of the peak data showed 4 Br sites in an asymmetric unit. The MAD phase calculation and solvent flattening were performed by SOLVE package. The final figure-of-merit was about 0.4 for the data at the resolution of 20-2.5 Å. The map was not so clear enough to build the model of the enzyme. We are now trying to get crystals with higher quality and to search the optimum soaking condition of Br for the next beam time.

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 the structure with 2 Ca^{2+} bound in the transmembrane high affinity sites and that in the absence of Ca^{2+} but in the presence of thapsigargin, a potent inhibitor.

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.

Therefore these crystals offer a unique opportunity to study the lipid-protein interactions in a great detail. Also, by solving the crystal structure of the Ca^{2+}-ATPase in the absence of Ca^{2+}, we showed that the transmembrane helices undergo dramatic rearrangements with surprisingly large components normal to the membrane (*Nature* 418: 605-611, 2002). Therefore, it is interesting to know how the lipid bilayers behave in the two crystals.

To visualise the bilayers, we need data for very low-resolution reflections that are neglected in the ordinary crystallographic analyses. The phase information for such low resolution reflections cannot be obtained reliably by multiple isomorphous replacements. Alternatively, we can use anomalous solvent as the contrast medium and MAD informaion as proposed by Fourme (*Acta Cryst*. D56: 1288-1303, 2000). Aurothioglucose, which has been used in electron microscopy for contrast variation, can be used for this purpose.

In previous trials, we used crystals of Ca^{2+}-ATPase with bound Mg/F, a phosphate analogue, because they are the most reproducible. In contrast, the crystals of Ca2+-bound form ($E1Ca^{2+}$) is the most difficult one to reproduce. Fortunately, we now know that the crystals of AlFx bound form in the presence of Ca^{2+} are easy to make. The concentration of aurothioglucose was 15%, which was the highest concentration with insignificant changes in the unit cell dimensions with the Mg/F crystals; the concentration could be 20% with the Al/F crystals. Therefore, in this term, we tried to collect the data from the Al/F crystals.

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. A complete set of MAD data consisting of 4 data sets around this wave length were collected from one crystal. Data merging went out successfully using Denzo and Scalepack. 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, a helium path appears to be mandatory, and we are designing one for this purpose. Also, it is advantageous to know the frames on which lowest resolution spots are expected to be recorded. Then, in the first run a beam stop may be inserted at a shortest distance from the specimen; then in the second run, we should be able to collect such frames again without the beam stop (with only a small beam stop in front of the imaging plates). We are developing such a program as well.