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Crystal structure of new lectin family and high mannose saccharide complex

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Lectin from alga forms new family, which only recognizes high mannose saccharide but not single saccharide. Recognition between proteins and carbohydrates is of prime importance in many biological processes. Legume lectins are well-studied proteins because they are not only easy to purify in large quantities but also exhibit a wide variety of carbohydrate specificities despite strong sequence conservations. But lectins from algae are quite hard to purify and even its existence was not known for a long time. Kanji Hori *et al.* first succeeded the purification of algae lectin, and now the number of species is over 20. Lectin from algae was also found to have the anti-cancer effect in the medical experiments for the mouse.

In the previous study using this beamline, we could determine the crystal structure of this high mannose saccharide specific lectin (ESA-2 from *Eucheuma Serra*) by multiple isomorphous replacement method. The three-dimensional structure of ESA-2 is a unique structure, which is not found in the lectin from high-plant. By comparing he primary sequences of lectins from other algae, and 4-folds of the self-primary structure, the saccharide binding site was clearly predicted. But because of this family of lectin is quite unique in the viewpoint of only specific for high mannose saccharide, the atomic interaction should be identified through the crystallographic method expecting the contribution of studying the lectin s physiological functions.

Because of the substrate saccharide of ESA-2 is quite expensive, we first tried soaking method to find the lectin-saccharide interaction. The sacchride-free crystal is quite thin as less than 10 μm. Thus, these thin plate crystals were soaked into the saturated sacchride solution. After 7 to 8 hours, the surface of crystal became dirty. This phenomenon supposed to be the effect of the strong interaction between protein and saccharide.

We have collected diffraction data sets of the soaking crystals. Their soaking times were 1, 2, 3, 3.5, 4, 6, and 7 hours. The crystals of 6 and 7 hours soaking were strongly decayed against the X-ray injection beam. Diffraction images were digitalized and merged using the program HKL2000 / SCALEPACK. But we could not find out the saccride electron density on the difference Fourier map, despite of the strong interaction. Thus, co-crystallization is now under way.

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Crystallographic Studies of ATP sulfurylase

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ATP sulfurylases (EC 2.7.7.4, sulfate adenylyltransferase) are ubiquitous enzymes that catalyse the primary step of sulfate activation. The reaction of inorganic sulfate with ATP to form adenosine-5'-phosphosulfate (APS) and pyrophosphate (PPi). ATP sulfurylase is used to generate APS from inorganic sulfate and ATP. This is the first step in the conversion of inorganic sulfate to a variety of organic sulfur compounds including sulfate esters in animals and reduced sulfur-containing biomolecules in plants and microorganisms.

The reconbinat ATP sulfurylase from *thermus thermophilus* was prepared using E.coli overexpression system. The Purified ATP sulfurylase was crystallized using hanging drop vapor diffusin meehod at 20℃. Crystals with dimensions of 0.1 x 0.1 x 0.05 mm^3 have been obtained in a few days. Se-Met substisuted crystal for the MAD phasing was crystallized under same conditions.

X-ray diffraction measurements were carried out under cryogenic condition (100K) using flash-cooling technique. The Se-Met crystal belongs to space group $P2_1$ with cell dimensions of a = 68.7 Å, b = 61.4 Å, c = 129.5 Å, α =90°, β =96.1°, γ =90°. MAD data with three wavelengths were collected up to 3.0 Å resolution using ADSC Quantum4R CCD detector. The MAD data were processed using the program HKL2000 for integration and scaling. Phase detemination is under progress.