Crystal Structure Analysis of CbnR: a LysR-type transcription regulator protein

S. Muraoka[1,3] (4445), R. Natsume[2] (5743), A. Okuta[3] (2140), M. Senda[3] (7967), and T. Senda*[3] (5700)

[1]Department of BioEngineering, Nagaoka University of Technology, [2]Faculty of Agriculture, The University of Tokyo, [3]Japan Biological Information Center, National Institute of Advanced Industorial Science and Technology

LysR-type transcriptional regulators are the most common types of positive regulators in prokaryotes. CbnR, one of the LysR-type transcriptional regulators, regulates the *cbnABCD* operon in *Ralstonia eutrophus* NH9, which encodes the enzymes responsible for the degradation of chlorocatechol. It has been found that CbnR specifically binds to the promoter of the *cbnA* gene and positively regulates the transcription of *cbnABCD* in the presence of inducers. Not much is known about the three-dimensional structure of these LysR-type transcriptional regulators. Only, the structure of the co-factor binding fragment of the LysR family member, CysB, has been found in the literature. In order to elucidate the structure-function relationship of the LysR-type transcriptional regulators, we solved the crystal structure of full-length CbnR. This is the first crystal structure of full-length LysR-type transcriptional regulators.

The crystal structure of CbnR was initially solved at 2.7-angstrom resolution by the MAD method. Then the crystallographic refinement was carried out with 2.2Å diffraction data collected at BL40B2 of SPring-8.

The CbnR subunit is composed of two domains, the DNA-binding and inducer-binding domains, which are connected by a long alpha helix with ca. 30 amino acid residues. The DNA-binding domain, which is the N-terminal part of the molecule, consists of three alpha helices and two antiparallel beta strands. The inducer-binding domain has essentially the same fold as that of CysB. CbnR was a homo-tetramer, which can be regarded as a dimer of the dimer that is comoposed of two subunits in different conformations, the compact and extended forms. The DNA-binding domains are located at the bottom of the main body of the tetramer. The arrangement of the DNA-binding domains seems to be suitable for interacting a promoter sequence with 60 bp.

Crystal structure of new lectin family and high mannose saccharide complex

Katsuo Katayanagi* (3688), Yasunori Kamiya (4895), Masaki Kawamoto (5820), Yoshihiko Hori (7392), Kenji Yamamoto (8594)

Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Kagamiyama 1-3-1, Higashi-Hiroshima 739-8526, Japan

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 sacchride 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.