Crystal structure analysis of reaction intermediates of LigAB, A protocatechuate 4,5-dioxygenase

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Background

LigAB is a class III catecholic extradiol type ring cleavage dioxygenase. In order to gain an insight into the catalytic mechanism of the enzyme, we determined the crystal structure of the LigAB at 2.2Å resolution [1]. In addition to this, we determined the crystal structure of LigAB in complex with its ligand, protocatechuate. These crystal structures have revealed that the structure of the active site of LigAB is very similar to that of a distinct type of extardiol type dioxygenase, the BphC enzyme, suggesting a convergent evolution between them [1].

So far the catalytic mechanism of the extradiol type dioxygenase has mainly been analyzed by the spectroscopic methods. However, it is difficult to obtain comprehensive structural information around the active site by the spectroscopic methods. In order to gain a precise insight into the catalytic mechanism of the extradiol type catecholic dioxygenases, we have tried to obtain a high-resolution crystal structure of LigAB in substrate free and complex forms. Here we report the crystal structure determination of LigAB at 1.5Å resolution.

Experimental

LigAB was expressed using *E.coli* and purified as described before [2]. Crystallization was carried out by the hanging drop vapor diffusion method. The obtained crystal was soaked in the standard buffer containing a cryo-protectant, mounted to a cryo-loop and frozen for data collection. Data collection was carried out at BL40B2 of SPring-8 using a CCD detector (ADSC). The collected data were processed using the program MOSFLM, and scaled with the program SCALA. The collected data had an R-merge value of 4.4% with the data completeness of 87.8% at 1.5Å resolution. Crystal structure of LigAB was determined by the molecular replacement method with the program MOLREP in the CCP4 program suite, and refined with the program REFMAC5. The current R-factor is 16.01% at 1.5Å resolution (Free R-factor = 18.4%).

References

[1] Sugimoto, K., Senda, T., Aoshima, H., Masai, E., Fukuda, M. & Mitsui, Y. (1999) *Structure* **7**, 953-965.
[2] Sugimoto, K., Senda, T., Masai, E., Kimbara, K., Fukuda, M. & Mitsui, Y. (1999)*Protein and Peptide Lett.* **6**, 403-406.

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

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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 eutropha*. The *cbnABCD* operon encodes for enzymes responsible for the degradation of chlorocatechol leading to the intermediates of the TCA cycle. It has been found that CbnR specifically binds to the promoter of CbnA and thereby positively regulating the transcription of *cbnABCD* in the presence of inducers like chlorocatechol.

The LysR family members have a high percentage of sequence identity in the N-terminal halves. There is a conserved helix-turn-helix DNA binding motif present in these N-terminal halves. 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 have tried to solve the crystal structure of CbnR.

The CbnR and selenomethionyl CbnR (Se-CbnR) were over expressed using *E.coli* and purified to near homogeneity by 2-step column chromatography. Crystallizations of CbnR and Se-CbnR were carried out using the typical hanging drop vapor diffusion method. The crystals were grown to the dimensions of *ca.* 0.2 x 0.03 x 0.03 mm^3.

To solve the structure of CbnR by MAD method, the absorption edge of selenium in the Se-CbnR crystal was determined by measuring the XAFS signal. The MAD data, however, could not be obtained, as the quality of the crystal was rather poor. Only a preliminary data set could be collected at 100K using the ADSC 2x2 CCD camera mounted on the beam line BL40B2. The wavelength was set to 0.9793 Å (peak). The data was processed using the program DPS/MOSFLM, resulting in the completeness of 81.6% and R_{merge}(I) of 13.0% at 4.2 Å resolution.

The improvement of the crystallization condition is underway to obtain larger crystals.