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Crystal structure analysis of the BphC-2,3-DHBP complex at 1.45Å resolution

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Introduction

Extradiol type catecholic ring cleavage dioxygenases (hereafter extradiol type dioxygenase) are key enzymes in the degradation pathways of aromatic compounds such as benzene, toluene and polychlorinated biphenyls (PCBs). To date, various types of extradiol type dioxygenases have been cloned from microorganisms such as Pseudomonads. These enzymes catalyze the addition of two atomic oxygens to the catechol ring of the substrate resulting in the cleavage of the catechol ring. These extradiol type dioxygenases typically contain one non-heme iron (Fe(II)) in their active site. The KKS102 BphC enzyme (hereafter BphC) is one of the extradiol type dioxygenases. Our crystal structure analysis of BphC [1, 2] has revealed that it is composed of eight identical subunits, each of which is related by the *422*-point group symmetry. Each subunit of BphC consists of two domains, domain 1 and domain 2. Each domain consists of two βαβββ motifs, thus, the subunit is composed of four βαβββ motifs. The Fe(II) ion, which is essential for the enzymatic reaction, is located inside the barrel-like structure of domain 2, and coordinated by His145, His209 and Glu260.

Recently, we have determined 2.0Å resolution crystal structure of BphC in complex with a substrate, 2,3-dihydroxybipheny (2,3-DHBP), using the active form enzyme under anaerobic condition [3]. The average coordination distance between the Fe ion and ligand atoms, however, showed a significant difference from that derived from the XAS analyses of a closely related protein. In order to gain a detailed insight into the structure of the active site, we have tried to solve a high-resolution structure of the BphC enzyme in complex with 2,3-DHBP.

Method

The BphC enzyme was purified as described earlier [2]. The purified enzyme was reactivated by adding ferrous ion. The active form enzyme was crystallized under strong magnetic field. The crystal of the BphC-2,3-DHBP complex was prepared by soaking method. The obtained crystal was mounted on a cryo-loop and flash-frozen in liquid nitrogen. Data collection was carried out using the ADSC CCD detector of BL6A at PF. All these experiments but the purification were carried out under anaerobic conditions. Crystal structure of the BphC-2,3-DHBP complex has been refined using the program CNS.

Results

Crystal structure of the BphC-2,3-DHBP complex has been refined at 1.45Å resolution, resulting in an R-factor of 20.0 % (free R-factor = 20.8%). The average coordination distance between the Fe ion and the ligand atoms in the present crystal structure is nearly the same as that derived from the XAS analysis. The difference between the two is less than 0.05Å, which is much smaller than the estimated coordinate error (0.17Å). It should be noted that the obtained coordination geometry is also nearly the same as that of a model compound of the extradiol type dioxygenase.

Comparison of the crystal structures between the substrate free and the complex forms has revealed that several conformational changes occur around the active site upon substrate binding. His194, the catalytic base of BphC, shifts toward the substrate by *ca.* 0.7Å, resulting in the formation of a hydrogen bond between the NE atom of Hi194 and the hydroxyl group (OH(3)) of the substrate. The hydrogen bond seems to facilitate a proton transfer between them.

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References

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