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Crystal structure of the terminal oxyngenase component of biphenyl dixoygenase derived from Rhodococcus sp. strain RHA1

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Introduction

The oxidative degradation pathways of aromatic compounds have been identified for many microbes. The first step of the degradation is dioxygenation of an aromatic ring by a multi-component dioxygenase, which is composed of ferredoxin reductase, ferredoxin, and terminal oxygenases. The terminal oxygenase component, which contains one Rieske cluster and one mononuclear iron in a subunit, catalyzes the stereospecific dioxygenation of aromatic compounds using a dioxygen and two electrons. The electrons, which are originally derived from NADH, are transferred to the dioxygenase from an electron transfer system composed of ferredoxin reductase and ferredoxin.

Biphenyl dioxygenase (hereafter BDO) is a dioxygenase that has been studied intensively. Most of the BDOs have been isolated from the biphenyl/polychlorinated-biphenyl (PCB) degradation pathways in PCB-degrading bacteria such as *Pseudomonas pseudoalcaligenes* KF707, *Burkholderia xenovorans* LB400, *Pseudomonas* sp. strain KKS102, and *Rhodococcus* sp. strain RHA1. These bacteria have attracted the attention of researchers because they promise to be useful in the bio-remediation of PCBs and other xenobiotic compounds. To utilize these bacteria for bio-remediation, however, the substrate specificity of these enzymes should be improved. Because the substrate specificity of BDO determines the whole degradation pathway, continuing efforts have been made to better understand the mechanism determining the substrate specificity of the enzymes. To reveal the mechanism of the substrate specificity of BDOs on the basis of the crystal structure, we have started the crystal structure analysis of BDO, which is derived from *Rhodococcus* sp. strain RHA1.

Results

RHA1 BDO was overexpressed using *Rhodococcus* sp. RHA1 and purified with two chromatographic steps. The obtained BDO was crystallized by the hanging drop vapor diffusion method using PEG4000 as a precipitant. [1] Crystals of the BDO-biphenyl complex were prepared by the co-crystallization method. First, biphenyl was dissolved in ethanol (10 mg/mL). The biphenyl solution was then added to the reservoir solution that is used in crystallizing the substrate-free form in 2% concentration. Droplets were prepared by mixing protein solution (10 mg/mL) and the reservoir solution in a 1:1 ratio. The reservoir condition of the co-crystallization is the same as that used in the substrate free form. [1] The obtained crystals of the BDO-biphenyl complex were isomorphous with those of the substrate-free form.

Data collections of BDO in substrate free and complex forms were carried out using the synchrotron radiation of BL6A of PF with an ADSC CCD camera. All the diffraction data were processed and scaled with the programs MOSFLM and SCALA in the CCP4 program suite, respectively.

The crystal structure of BDO was determined with the molecular replacement method using the program AMORE in the CCP4 program suite. The crystal structure of NDO (naphthalene dioxygenase), which has an approximately 32% sequence identity with RHA1 BDO, was used as a search model. The solution derived from a cross rotation search was assessed using the results of a self-rotation search. The enantiomorph of the crystal was determined as $P4_32_12$ in the course of a translational search. Crystallographic refinement was carried out using the programs CNS and REFMAC, resulting in the final R-factor of 16.7%, and free R-factor of 20.1% at 2.2Å resolution.

The crystal structure of the BDO-substrate copmlex was determined by the difference Fourier method, because the crystal of the substrate complex is nearly isomorphous to that of the BDO in the substrate-free form. Crystallographic refinement was carried out using the programs CNS and REFMAC, resulting in the final R-factor of 18.4%, and free R-factor of 23.4% at 2.6Å resolution.

These crystal structures revealed that the substrate-binding pocket makes significant conformational changes upon substrate binding to accommodate the substrate into the pocket. Our analysis of the crystal structures suggested that the residues in the substrate-binding pocket can be classified into three groups, which respectively seem to be responsible for the catalytic reaction, the orientation/conformation of the substrate, and the conformational changes of the substrate-binding pocket. The cooperative actions of residues in the three groups seem to determine the substrate specificity of the enzyme.

References

[1] V. Nagarajan et al., Protein Pept Lett. 10, 412 (2003).

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