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Crystallographic analysis of the Mn(II)-dependent 2,3-dihydroxybiphenyl 1,2-dioxygenase, BphC_JF8

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Introduction

Extradiol-cleaving dioxygenases play a key role in the degradation pathways of aromatic compounds. These enzymes typically contain a non-heme iron (Fe(II)) in their active site. Because the Fe(II) ion is easily oxidized by dioxygen, most of these enzymes are unstable under aerobic conditions. The industrial utilization of these enzymes is therefore difficult.

BphC_JF8 derived from *Bacillus* sp. strain JF8, which shows high amino-acid sequence similarity to the typical extradiol-cleaving dioxygenases, contains a Mn(II) ion at the active site[1]. BphC_JF8 is stable under aerobic conditions, because Mn(II) is not oxidized by dioxygen. Interestingly, BphC_JF8 can also bind Fe(II) at the active site, forming an active enzyme. Since typical extradiol-cleaving dioxygenases bind only an Fe(II) ion at the active site, a comparison of the active site structures of BphC_JF8 and other typical extradiol-cleaving dioxygenases, such as BphC derived from *Acidovorax* sp. strain KKS102 [2], would provide a novel insight into the ion selectivity of the enzyme. We therefore attempted to determine the high-resolution crystal structure of BphC_JF8. Potentially, the mechanism of the ion selectivity of the extradiol-cleaving dioxygenses could be utilized to design air-stable Mn(II)-containing dioxygenases from typical Fe(II)-containing dioxygenases.

Here, we report the crystal structure analysis of the Mn(II)- and Fe(II)- binding forms of BphC_JF8.

Methods

Purification of BphC_JF8 was carried out as described by Hatta *et al*. [1]. BphC_JF8–Fe(II) was crystallized under anaerobic conditions to avoid the oxidation of an Fe(II) ion. BphC_JF8–Mn(II) was also crystallized under anaerobic conditions. For anaerobic crystallization, we utilized an anaerobic chamber system developed in our laboratory [3]. The crystals grew to their full size in 2 weeks with approximate dimensions of 1.0 x 0.5 x 0.03 mm^3. The crystals were frozen using liquid nitrogen in the anaerobic chamber. The frozen crystals were stored in a dry shipper (SC 4/3V, MVE) to transport them to the Photon Factory.

Results

High-resolution diffraction data of the BphC_JF8 crystals were collected at beam lines BL-5A, BL-17A and NW12A. The diffraction data were processed using an *XDS* or *HKL2000* program (Table 1). The crystal structure of BphC_JF8–Mn(II) and BphC_JF8–Fe(II) were determined by the molecular-replacement method using program *MOLREP* from the *CCP4* program suite. Crystallographic refinements of BphC_JF8–Mn(II) and BphC_JF8–Fe(II) are in progress.

Table 1 Data-collection statistics

Crystal form	BphC_JF8-Mn(II)	BphC_JF8-Fe(II)
X-ray source	Photon Factory	Photon Factory
Beamline	BL-5A	BL-5A
Oscillation angle (°)	0.3	0.3
Exposure time (s)	1	2
Wavelength (Å)	1.0000	1.0000
Temperature (K)	100	100
Space group	*P*1	*P*1
Unit-cell parameters (Å, °)	*a*=62.8, *b*=71.5, *c*=94.0, α=71.2, β=81.1, γ=63.9	*a*=63.1, *b*=71.2, *c*=94.0, α=71.3, β=81.1, γ=63.6
Resolution (Å)	17.0-1.14 (1.21-1.14)	15.0-1.35 (1.42-1.35)
Observations	2,787,206 (226,918)	877,426 (122,042)
Unique reflections	466,255 (71,612)	285,864 (39,623)
Completeness (%)	91.9 (86.2)	93.6 (92.1)
Redundancy	6.0 (3.2)	3.1 (3.1)
Average $I/\sigma(I)$	12.13 (3.15)	10.91 (4.88)
Rmerge (%)	0.080 (0.377)	0.071 (0.296)

Values in parentheses are for the outermost resolution shell.

References

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