

Crystallographic analysis of the ferredoxin reductase, BphA4, during the catalysis

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Introduction

The oxidative degradation of biphenyl/PCBs by *Pseudomonas* sp. strain KKS102 begins with hydroxylation of an aromatic ring by a multi-component dioxygenase, BphA. This enzyme is composed of ferredoxin reductase, ferredoxin and terminal oxygenase. BphA4 is the ferredoxin reductase component of BphA, whose crystal structure was solved at 2.3Å by our group five years ago [1]. Since then, we have tried to determine the high-resolution crystal structures of the reaction intermediates of BphA4 to gain a deeper insight into the electron transport mechanism between ferredoxin and BphA4. Here we report the high-resolution data collection of BphA4 in oxidized form, its NADH complex forms (reduced and blue semiquinone forms) and reoxidized form.

Method

The purification and crystallization of BphA4 were carried out as described by Yamada *et al.* [2]. The crystals grew to their full size in 3-5 days with approximate dimensions of 1.0x0.5x0.3 mm³. Crystals of the BphA4-NADH complex were prepared by the soaking method. The crystal color gradually changed from yellow to blue in aerobic NADH solution, and its color gradually changed from yellow to light yellow in anaerobic NADH solution. The crystal was then flash-frozen.

Result

Data collections of the reaction intermediate crystals of the BphA4 were carried out on the beam lines NW12, BL5A and BL18B. The diffraction data were processed and scaled using the program HKL2000. The data collection statistics are given in Table 1. The crystal structures of these reaction intermediates were determined by the molecular replacement method. Crystallographic refinements of these structures are in progress.

References

- [1] T. Senda *et al.*, *J. Mol. Biol.*, **304**, 397-410 (2000).
 [2] T. Yamada *et al.*, *Protein Pept. Letters*, **7**, 277-280 (2000).

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Table 1 Summary of crystallographic data collection and processing

Crystal form	oxidized	reduced
Space group	<i>P</i> 6 ₂ 2	<i>P</i> 6 ₂ 2
Unit-cell parameters (Å)	<i>a</i> = <i>b</i> =98.4, <i>c</i> =170.5	<i>a</i> = <i>b</i> =98.1, <i>c</i> =170.3
X-ray source	Photon Factory	Photon Factory
Beamline	NW12	NW12
Wavelength	1.0000	0.9798
Temperature (K)	100	100
Resolution (Å)	50-1.45	50-1.95
Mosaicity (°)	0.5	0.5
No. of observations	766,041	382,415
Unique reflections	86,550	35,836
Completeness	100.0	99.2
Multiplicity	4.4	10.7
Overall I/σ	7.2	13.9
Rsym (%)	7.0	4.2
Rmerge (%)	7.8	5.9

Crystal form	blue-semiquinone	re-oxidized
Space group	<i>P</i> 6 ₂ 2	<i>P</i> 6 ₂ 2
Unit-cell parameters (Å)	<i>a</i> = <i>b</i> =98.4, <i>c</i> =170.2	<i>a</i> = <i>b</i> =98.0, <i>c</i> =170.1
X-ray source	Photon Factory	Photon Factory
Beamline	BL5A	BL18B
Wavelength	0.9780	1.3000
Temperature (K)	100	100
Resolution (Å)	50-1.7	50-2.0
Mosaicity (°)	0.3	0.5
No. of observations	1,120,578	231,153
Unique reflections	53,034	33,024
Completeness	97.9	98.7
Multiplicity	21.1	7.0
Overall I/σ	13.8	12.5
Rsym (%)	4.2	5.5
Rmerge (%)	5.2	7.1