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# 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 mm<sup>3</sup>. 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**

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

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Table 1	Summary	of crystall	lographic data	a collection and
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Crystal form         oxidized         reduced           Space group $P6_122$ $P6_22$ Unit-cell $a=b=98.4$ , $a=b=98.1$ ,           parameters (Å) $c=170.5$ $c=170.3$ X-ray source         Photon Factory         Photon Factory           Beamline         NW12         NW12           Wavelength         1.0000         0.9798           Temperature         100         100           (K)         100         100           Resolution (Å)         50-1.45         50-1.95           Mosaicity (°)         0.5         0.5           No.of         766,041         382,415           observations         100.0         99.2           Multiplicity         4.4         10.7           Overall I/ $\sigma$ 7.2         13.9           Rsym (%)         7.0         4.2           Rmerge (%)         7.8         5.9		processing	
Space group $P6_{1}22$ $P6_{1}22$ Unit-cell $a=b=98.4$ , $a=b=98.1$ ,           parameters (Å) $c=170.5$ $c=170.3$ X-ray source         Photon Factory         Photon Factory           Beamline         NW12         NW12           Wavelength         1.0000         0.9798           Temperature         100         100           (K)         100         100           Resolution (Å)         50-1.45         50-1.95           Mosaicity (°)         0.5         0.5           No.of         766,041         382,415           observations         100.0         99.2           Multiplicity         4.4         10.7           Overall I/ $\sigma$ 7.2         13.9           Rsym (%)         7.0         4.2           Rmerge (%)         7.8         5.9	Crystal form	oxidized	reduced
Unit-cell $a=b=98.4$ , $c=170.5$ $a=b=98.1$ , $c=170.3$ parameters (Å) $c=170.5$ $c=170.3$ X-ray sourcePhoton FactoryPhoton FactoryBeamlineNW12NW12Wavelength1.00000.9798Temperature (K)100100Resolution (Å)50-1.4550-1.95Mosaicity (°)0.50.5No.of observations766,041382,415Unique reflections86,55035,836Completeness100.099.2Multiplicity4.410.7Overall I/ $\sigma$ 7.213.9Rsym (%)7.85.9	Space group	P6,22	P6,22
parameters (Å) $c=170.5$ $c=170.3$ X-ray sourcePhoton FactoryPhoton FactoryBeamlineNW12NW12Wavelength1.00000.9798Temperature (K)100100(K)100100Resolution (Å)50-1.4550-1.95Mosaicity (°)0.50.5No.of observations766,041382,415Unique reflections86,55035,836Completeness100.099.2Multiplicity4.410.7Overall I/ $\sigma$ 7.213.9Rsym (%)7.04.2Rmerge (%)7.85.9	Unit-cell	<i>a</i> = <i>b</i> =98.4,	<i>a</i> = <i>b</i> =98.1,
X-ray source         Photon Factory         Photon Factory           Beamline         NW12         NW12           Wavelength         1.0000         0.9798           Temperature         100         100           (K)         100         100           Resolution (Å)         50-1.45         50-1.95           Mosaicity (°)         0.5         0.5           No.of         766,041         382,415           observations         86,550         35,836           Completeness         100.0         99.2           Multiplicity         4.4         10.7           Overall I/ $\sigma$ 7.2         13.9           Rsym (%)         7.0         4.2           Rmerge (%)         7.8         5.9	parameters (Å)	c=170.5	c=170.3
$\begin{array}{c c c c c c c } \hline Beamline & NW12 & NW12 \\ \hline Wavelength & 1.0000 & 0.9798 \\ \hline Temperature & 100 & 100 \\ \hline (K) & 50-1.45 & 50-1.95 \\ \hline Mosaicity (°) & 0.5 & 0.5 \\ \hline No.of & 766,041 & 382,415 \\ \hline Unique & 86,550 & 35,836 \\ \hline Completeness & 100.0 & 99.2 \\ \hline Multiplicity & 4.4 & 10.7 \\ \hline Overall I/\sigma & 7.2 & 13.9 \\ \hline Rsym (\%) & 7.0 & 4.2 \\ \hline Rmerge (\%) & 7.8 & 5.9 \\ \hline \end{array}$	X-ray source	Photon Factory	Photon Factory
Wavelength1.0000 $0.9798$ Temperature (K)100100Resolution (Å)50-1.4550-1.95Mosaicity (°)0.50.5No.of observations766,041 $382,415$ Unique reflections86,550 $35,836$ Completeness100.099.2Multiplicity4.410.7Overall I/ $\sigma$ 7.213.9Rsym (%)7.04.2Rmerge (%)7.85.9	Beamline	NW12	NW12
$\begin{array}{c c c c c c c c } \hline Temperature & 100 & 100 \\ \hline Resolution (Å) & 50-1.45 & 50-1.95 \\ \hline Mosaicity (°) & 0.5 & 0.5 \\ \hline Mosaicity (°) & 0.5 & 0.5 \\ \hline Mosof & 766,041 & 382,415 \\ \hline observations & 766,041 & 382,415 \\ \hline Unique & 86,550 & 35,836 \\ \hline Completeness & 100.0 & 99.2 \\ \hline Multiplicity & 4.4 & 10.7 \\ \hline Overall I/\sigma & 7.2 & 13.9 \\ \hline Rsym (\%) & 7.0 & 4.2 \\ \hline Rmerge (\%) & 7.8 & 5.9 \\ \hline \end{array}$	Wavelength	1.0000	0.9798
(K)         100         100           Resolution (Å)         50-1.45         50-1.95           Mosaicity (°)         0.5         0.5           No.of         766,041         382,415           Unique         86,550         35,836           reflections         100.0         99.2           Multiplicity         4.4         10.7           Overall I/ $\sigma$ 7.2         13.9           Rsym (%)         7.0         4.2           Rmerge (%)         7.8         5.9	Temperature	100	100
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	_(K)	100	
$\begin{array}{c c c c c c c c } Mosaicity (°) & 0.5 & 0.5 \\ \hline No.of & 766,041 & 382,415 \\ \hline observations & 86,550 & 35,836 \\ \hline Completeness & 100.0 & 99.2 \\ \hline Multiplicity & 4.4 & 10.7 \\ \hline Overall I/\sigma & 7.2 & 13.9 \\ \hline Rsym (\%) & 7.0 & 4.2 \\ \hline Rmerge (\%) & 7.8 & 5.9 \\ \hline \end{array}$	Resolution (Å)	50-1.45	50-1.95
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/o         7.2         13.9           Rsym (%)         7.0         4.2           Rmerge (%)         7.8         5.9	Mosaicity (°)	0.5	0.5
observations         700,041         382,413           Unique reflections         86,550         35,836           Completeness         100.0         99.2           Multiplicity         4.4         10.7           Overall I/o         7.2         13.9           Rsym (%)         7.0         4.2           Rmerge (%)         7.8         5.9	No.of	766 0/1	382 415
Unique reflections         86,550         35,836           Completeness         100.0         99.2           Multiplicity         4.4         10.7           Overall I/o         7.2         13.9           Rsym (%)         7.0         4.2           Rmerge (%)         7.8         5.9	observations	700,041	562,415
reflections         80,550         55,650           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	Unique	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	reflections	00,550	
Multiplicity         4.4         10.7           Overall I/σ         7.2         13.9           Rsym (%)         7.0         4.2           Rmerge (%)         7.8         5.9	Completeness	100.0	99.2
Overall I/o         7.2         13.9           Rsym (%)         7.0         4.2           Rmerge (%)         7.8         5.9	Multiplicity	4.4	10.7
Rsym (%)         7.0         4.2           Rmerge (%)         7.8         5.9	Overall I/o	7.2	13.9
Rmerge (%)         7.8         5.9	Rsym (%)	7.0	4.2
	Rmerge (%)	7.8	5.9

Crystal form	blue- semiquinone	re-oxidized
Space group	P6,22	P6,22
Unit-cell	<i>a</i> = <i>b</i> =98.4,	<i>a</i> = <i>b</i> =98.0,
parameters (Å)	c=170.2	c=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/o	13.8	12.5
Rsym (%)	4.2	5.5
Rmerge (%)	5.2	7.1