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Crystallographic analysis of a BphA4 mutant

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1 Introduction

We have studied an electron transfer system of a multi-component dioxygenase, BphA, derived from *Acidovorax* sp. strain KKS102. BphA4, which is an FAD-containing NADH-dependent ferredoxin reductase, receives two electrons from NADH and delivers one electron each to ferredoxins (BphA3). In the present study, we tried a perfect conversion of co-factor specificity of BphA4 from NADH to NADPH. In order to change its co-factor specificity, target residues for mutation were selected on the basis of the crystal structure of the wild type (WT) BphA4-NAD^+ complex [1]. This structure showed that Glu175, Thr176, Gln177, Ser182 and Arg183 interacted with a ribose moiety of NAD^+. Of the five residues, three consecutive residues, Gln175, Thr176 and Gln177, are nearby located to 2'-OH of NAD^+. Therefore, these residues were selected as targets of mutation, and random mutagenesis was applied for them. As a result, mutant E175C/T176R/Q177G, hereafter CRG, showed high specificity to NADPH. In order to elucidate the molecular mechanism of $NADP^+$ binding, mutant CRG was crystallographically analyzed. Here we report a preliminary X-ray crystallographic analysis of the BphA4 CRG mutant.

2 Experiment

A mutant gene of BphA4 was prepared by the PCR mutagenesis method. Mutant CRG was expressed in *Escherichia coli* and was purified as described previously [2]. Crystallization and soaking experiments were performed under anaerobic conditions in order to avoid the oxidation of reduced FAD in BphA4 [3]. The BphA4 CRG mutant was crystallized by the sitting-drop vapour diffusion method. Yellow crystals appeared in 2.0 M sodium formate, 0.1 M sodium acetate pH 5.2. Obtained crystals were soaked in an artificial mother liquor (2.5 M sodium formate, 0.1 M sodium acetate pH 5.2) containing 10 mM NAD(P)H for 2 hours, and then crystals were transferred into a cryoprotectant solution (27.5%(v/v) glycerol, 0.1 M sodium acetate pH 5.2) for 20 seconds. The crystals were then frozen using liquid nitrogen.

3 Results and Discussion

Diffraction data of BphA4 CRG mutant crystals were collected at BL32XU of SPring-8 (Table 1). The diffraction data were processed and scaled using the program XDS and XSCALE, respectively. The crystals belonged to space group $P6_122$, which is the same as that of BphA4 WT crystals. The crystal structures were determined by the molecular replacement method by MOLREP using the structure of BphA4 WT as a search model. Crystallographic refinement is in progress.

Table 1 Crystallographic summary

Crystal form	E175C/T176R/ Q177G	E175C/T176R/ Q177G
Soaking condition	10 mM NADH, 2hr	10 mM NADPH, 2hr
X-ray source	SPring-8	SPring-8
Beamline	BL32XU	BL32XU
Oscillation angle (°)	0.5	0.5
Exposure time (s)	1	1
Wavelength (Å)	0.97934	0.97934
Temperature (K)	95	95
Space group	$P6_122$	$P6_122$
Unit-cell parameters (Å)	a=b=98.2, c=171.3	a=b=98.2, c=171.0
Resolution (Å)	50.0-1.70 (1.79-1.70)	50.0-1.75 (1.84-1.75)
Unique reflections	101,249 (14,531)	93,099 (12,974)
Completeness (%)	99.6 (99.9)	100.0 (100.0)
Redundancy	5.6 (5.7)	11.5 (11.4)
Average $I/\sigma(I)$	14.1 (3.3)	22.9 (4.2)
Rmerge (%)	0.075 (0.553)	0.067 (0.610)

Values in parentheses are for the outermost resolution shell.

References
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