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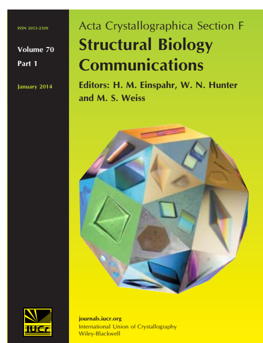

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Purification, crystallization and preliminary X-ray crystallographic studies of FMN-bound and FMN-free forms of aromatic acid decarboxylase (CpsUbiX) from the psychrophilic bacterium *Colwellia psychrerythraea* 34H

Hackwon Do,[a,b] Chang Woo Lee,[a,b] Se Jong Han,[a,b] Sung Gu Lee,[a,b] Hak Jun Kim,[c] Hyun Park[a,b] and Jun Hyuck Lee[a,b]*

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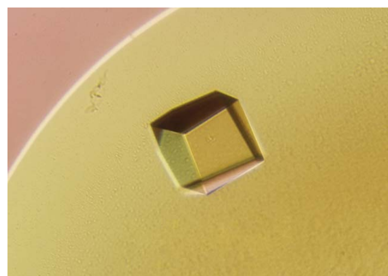
The *ubiX* gene (UniProtKB code Q489U8) of *Colwellia psychrerythraea* strain 34H has been annotated as a putative flavin mononucleotide (FMN)-dependent aromatic acid decarboxylase. Based on previous studies of homologous proteins, CpsUbiX is thought to catalyze the decarboxylation of 3-octaprenyl-4-hydroxybenzoate to produce 2-polyprenylphenol in the ubiquinone-biosynthesis pathway using a noncovalently bound FMN molecule as a cofactor. However, the detailed mechanisms of this important enzyme are not yet clear and need to be further elucidated. In this study, it was found that the V47S single mutation resulted in a loss of FMN binding, resulting in the production of FMN-free CpsUbiX protein. This mutation is likely to destabilize FMN–protein interactions without affecting the overall structural folding. To fully characterize the conformational changes upon FMN binding and the enzymatic mechanism at the molecular level, the wild-type (FMN-bound) and V47S mutant (FMN-free) CpsUbiX proteins were purified and crystallized using the sitting-drop vapour-diffusion method. Furthermore, complete diffraction data sets for FMN-bound (space group $C222_1$) and FMN-free (space group $P23$) forms were obtained to 2.0 and 1.76 Å resolution, respectively.

1. Introduction

Ubiquinone (coenzyme Q) is an essential compound because it is an obligatory component of the electron-transfer pathway in microorganisms (Blankenship & Parson, 1979). In the most well studied *Escherichia coli* system, several *ubi* gene clusters are known to encode a set of enzymes (UbiA, UbiB, UbiC, UbiD, UbiE, UbiF, UbiG, UbiH and UbiX) that catalyze the synthesis of ubiquinone from the compound chorismate. Based on genetic deletion studies, the *E. coli* UbiX enzyme is functionally involved in the decarboxylation step from 3-octaprenyl-4-hydroxybenzoate to 2-octaprenyl phenol (Knoell, 1979; Meganathan, 2001). This reaction is an FMN (flavin mononucleotide)-dependent reaction and thus UbiX belongs to the FMN-dependent decarboxylase family. The FMN cofactor plays central roles in electron transfer in various biological enzymatic processes. Interestingly, a recent report has shown that two completely different genes designated *ubiD* and *ubiX* encode two different decarboxylases (about 9% sequence identity) and they perform the same reaction in *E. coli*. However, both enzymes are essential for the normal production of ubiquinone in log-phase *E. coli* cells. Although UbiX has been annotated as a 3-octaprenyl-4-hydroxybenzoate carboxy-lyase, its enzyme activity has not been directly confirmed because of solubility problems arising from the membrane-bound natural substrate. Thus, the detailed decarboxylation mechanism and specific enzyme activity of UbiX remain unclear. To date, FMN-bound crystal structures of *Pseudomonas aeruginosa* UbiX (Kopec *et al.*, 2011) and *E. coli* Pad1 (a paralogue of UbiX; Rangarajan *et al.*, 2004) are known. These proteins share conserved FMN-binding residues and a common dodecamer oligomeric state.

We have studied *ubi* gene-cluster enzymes from the psychrophilic Gram-negative bacterium *Colwellia psychrerythraea* strain 34H (Methé *et al.*, 2005) to understand how the enzymes catalyze and regulate ubiquinone biosynthesis. In this study, we cloned, over-



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expressed and purified the *ubiX* gene product (UniProtKB code Q489U8; 206 amino-acid residues) using an *E. coli* expression system. Interestingly, the expressed wild-type CpsUbiX had a yellowish colour, and further spectroscopic analysis revealed that wild-type CpsUbiX had an FMN:protein molar ratio of 1.028:1. Moreover, we investigated the generation of FMN-free protein by mutagenesis to study how the FMN cofactor participates in the decarboxylation process. To this end, several point mutants were generated throughout the predicted FMN-binding site and the effect of the substitution was examined by spectroscopic analysis. The protein with a V47S single mutation could not bind FMN and had an FMN:protein molar ratio of 0.086:1. We also successfully crystallized and collected complete X-ray diffraction data sets for wild-type (FMN-bound) and V47S mutant (FMN-free) CpsUbiX proteins. We believe that the FMN-complexed and FMN-free CpsUbiX structures will be valuable to characterize the FMN-binding mode and enzymatic mechanism of this important enzyme.

2. Materials and methods

2.1. Cloning, expression and purification of wild-type CpsUbiX

The *ubiX* gene (UniProtKB code Q489U8) was amplified by the polymerase chain reaction (PCR) using *C. psychrerythraea* strain 34H genomic DNA (purchased from the ATCC) as a template with forward primer 5′-CGA TAA CAT ATG AAC AAT GAT TTT AAT GGT-3′ and reverse primer 5′-CGA TAA CTC GAG TCA AAT GTT ATA ACC CCA ACG-3′. The amplified DNA fragments were cleaved with *Nde*I and *Xho*I restriction enzymes and ligated into the multi-cloning site (MCS) of the pET-28a vector (Novagen, Madison, USA). The final construct had an additional 20 amino acids (MGSS*HHHHHH*SSG**LVPRGS**H) including the hexahistidine tag (italicized) and a thrombin protease recognition site (bold) at the N-terminus. The resulting plasmid was verified by DNA sequencing and was then transformed into *E. coli* strain BL21(DE3) for protein expression. The cells were grown in Luria–Bertani (LB) medium at 310 K with 50 µg ml^{-1} kanamycin until the OD_{600} approached 0.7; the cells were then induced using 1 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) at 298 K for 20 h. After induction, the cells were harvested by centrifugation (VS-24SMTi; Vision Scientific, Bucheon, Republic of Korea) at 7000 rev min^{-1} and 277 K for 30 min from 4 l culture and resuspended in cold lysis buffer (50 m*M* sodium phosphate, 300 m*M* NaCl, 5 m*M* imidazole pH 8.0) with 0.2 mg ml^{-1} lysozyme and 0.5 m*M* phenylmethylsulfonyl fluoride (PMSF). After incubation for 1 h at 277 K, the cells were disrupted using a Vibra-Cell VCX400 sonicator (Newtown, USA) on ice. The lysate was centrifuged again to remove cell debris at 16 000 rev min^{-1} for 1 h at 277 K, and the lysate was then poured into a gravity-flow column prepacked with Ni–NTA resin. The bound protein was washed with ten column volumes of wash buffer (50 m*M* sodium phosphate, 300 m*M* NaCl, 10 m*M* imidazole pH 8.0) and eluted with elution buffer (50 m*M* sodium phosphate pH 8.0, 300 m*M* NaCl, 300 m*M* imidazole). After cleavage of the hexahistidine tag at 277 K overnight using thrombin, purification by size-exclusion chromatography with a buffer consisting of 20 m*M* Tris–HCl pH 8, 150 m*M* NaCl, 1 m*M* DTT was performed using a Superdex 200 HiLoad 16/60 column (GE Healthcare, Piscataway, USA). The wild-type CpsUbiX was purified to homogeneity (about 87.8%), as judged from densitometer (Gel Doc EZ imager with *Image Lab* software; Bio-Rad) scans of the SDS–PAGE gel, and the protein was concentrated to 21 mg ml^{-1} using Amicon Ultra-15 centrifugal ultrafiltration (Millipore, Bedford, USA).

2.2. Mutagenesis, expression and purification of mutant (FMN-free) CpsUbiX

Site-directed mutagenesis was performed by PCR using the following mutagenic primer pairs (the V47S point-mutation site is shown in bold) with plasmids containing the wild-type CpsUbiX gene as template DNA: 5′-AGT GCC GGT CGT ATT **TCT** TTA GAT ACT GAA GTG-3′ and 5′-CAC TTC AGT ATC TAA **AGA** AAT ACG ACC GGC ACT-3′. The single-stranded oligonucleotides containing the point mutation were synthesized by and purchased from Bioneer (Daejeon, Republic of Korea). After amplification, the PCR fragments were digested by the *Dpn*I restriction enzyme to eliminate template plasmids. The presence of the single mutation was confirmed by DNA sequencing. The mutated plasmid was transformed into competent *E. coli* BL21(DE3) cells and the protein was overexpressed and purified using the same procedures as described for wild-type CpsUbiX. The yield of the mutant enzyme was 25 mg from 1 l culture and the purity (about 96.1%) was quantified by densitometric (Gel Doc EZ imager with *Image Lab* software; Bio-Rad) analysis of the SDS–PAGE gels. Protein concentrations were determined using UV spectrophotometry with an extinction coefficient at 280 nm of 19 360 M^{-1} cm^{-1} based on the amino-acid sequence with non-reduced cysteines.

2.3. Calculation of FMN:protein ratios

To determine the concentration of FMN associated with wild-type and V47S mutant CpsUbiX proteins, the spectrophotometric determination method was used with riboflavin 5′-monophosphate sodium salt hydrate (FMN) purchased from Sigma–Aldrich (St Louis, Missouri, USA). The FMN was prepared in water or in the buffer used for the enzyme solution. The standard curve was plotted as the concentration of FMN *versus* absorbance at 450 nm with serial dilution. Wild-type CpsUbiX and FMN were mixed in a molar ratio of 1:3 and purified by size-exclusion chromatography to confirm that the FMN was completely bound to the wild-type protein in buffer (20 m*M* Tris–HCl pH 8.0, 150 m*M* NaCl). The wild-type and V47S mutants were diluted and analyzed using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan) over a wavelength range of 250–600 nm at room temperature. The concentration of wild-type and V47S mutant proteins was measured using an extinction coefficient of 19 360 M^{-1} cm^{-1} after subtraction of the degree of absorption calculated using the standard curve of FMN at 280 nm. Because the absorbance of FMN at 280 nm was concentration-dependent, the concentration of FMN in each sample was calculated using a series of FMN standards at 450 nm instead of using deflavination from the proteins. The molar ratio of FMN to protein was expressed as FMN concentration to protein concentration. The calculated FMN:protein ratio was 1.028 for wild-type CpsUbiX and 0.086 for the V47S mutant protein.

2.4. Analytical size-exclusion chromatography

To estimate the oligomeric state and molecular mass of CpsUbiX, the purified protein samples were subjected to a Superdex 200 10/300 GL column from GE Healthcare connected to an ÄKTA FPLC system. The column was equilibrated in 50 m*M* phosphate buffer pH 7, 150 m*M* NaCl at a flow rate of 0.4 ml min^{-1}. The column was calibrated at 277 K with globular protein standards (MWGF1000, Sigma) that included apoferritin (443 kDa; V_e = 10.54 ml), β-amylase (200 kDa; V_e = 11.95 ml), albumin (66 kDa; V_e = 14.32 ml) and carbonic anhydrase (29 kDa; V_e = 16.72 ml). The void volume of the column (V_o) was obtained as 8.135 ml using blue dextran (2000 kDa)

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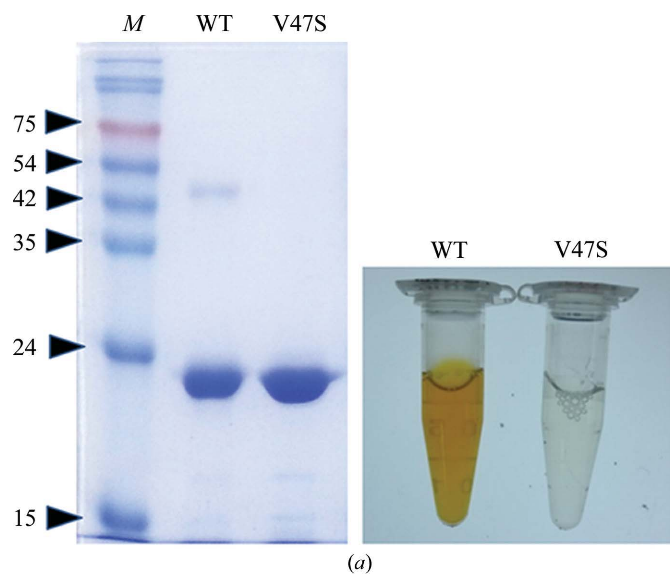
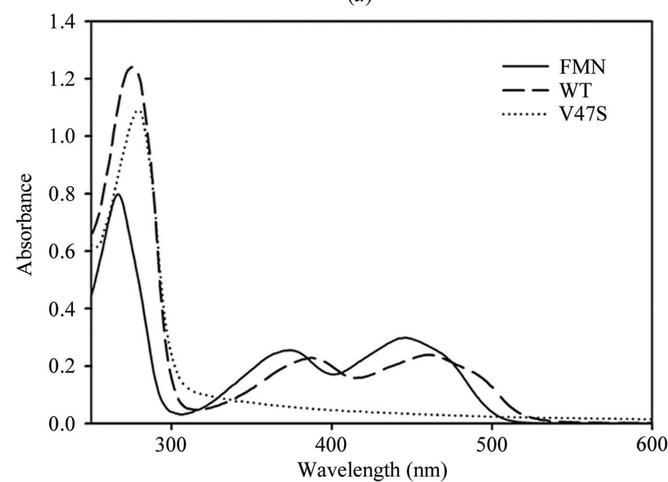
and V_e is the peak elution volume. The V_e/V_o values were used to generate a standard curve and to determine the molecular mass of CpsUbiX from the standard curve.

2.5. Circular-dichroism profiles of wild-type CpsUbiX and the V47S mutant

Circular-dichroism (CD) experiments were performed with protein samples (wild-type CpsUbiX and V47S mutant) at a concentration of 17.7 μ*M* in distilled water using a Chirascan CD spectrometer from Applied Photophysics (Leatherhead, England) connected to a Peltier temperature controller. Protein samples were placed in 1 mm path-length quartz cuvettes (Hellma, New York, USA) and spectra were collected from 190 to 260 nm at 293 K and 0.25 s per point. Ten spectra were averaged to give the final trace and blank spectra were subtracted. The secondary structures were analyzed from the CD spectra using the *CDNN* software (Böhm *et al.*, 1992).



(*a*)



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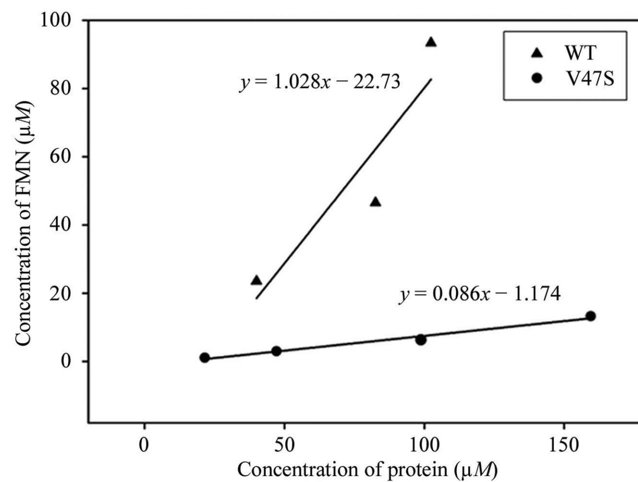

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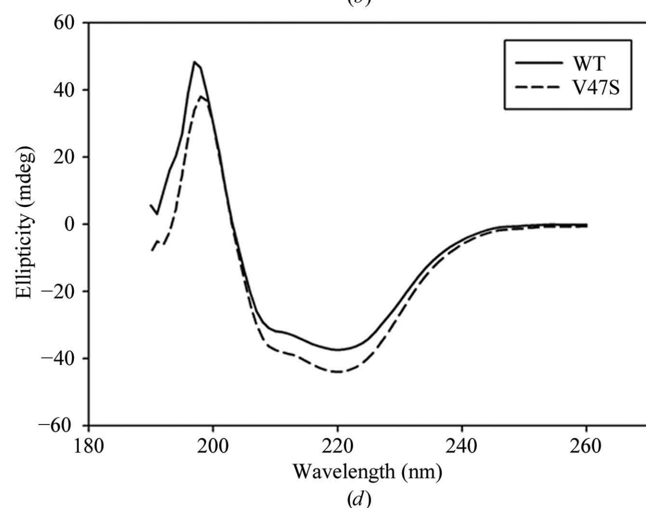

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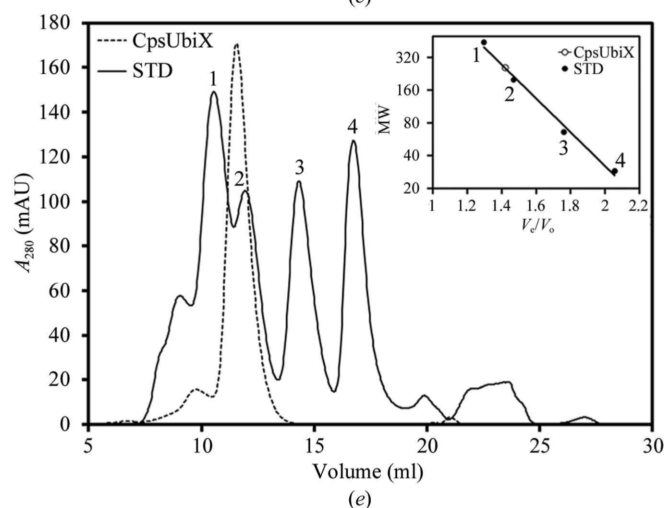

(*e*)

Figure 1

Purification and characterization of FMN-bound and FMN-free forms of CpsUbiX. (*a*) Wild-type (FMN-bound; WT) and V47S mutant (FMN-free) CpsUbiX proteins were loaded onto a 12% SDS–PAGE gel before the crystallization trials and the proteins were visualized by Coomassie Blue staining (left). Lane *M* contains molecular-mass marker (labelled in kDa). Wild-type CpsUbiX protein had a yellowish colour, while the V47S mutant did not (right). (*b*) Absorption spectra of the purified wild-type and V47S mutant CpsUbiX proteins. AU, absorbance units. Free FMN and wild-type CpsUbiX protein had an absorption maximum from 360 to 480 nm, while the V47S mutant did not show any absorbance in this region. (*c*) The FMN binding molar ratio was plotted and calculated at several different concentrations of protein sample using a 450 nm absorption value. See §2 for additional details. (*d*) CD spectra of the wild-type (solid line) and V47S mutant (dashed line) proteins of CpsUbiX. The concentration of both protein samples was 17.7 μ*M*. (*e*) Overlay of size-exclusion chromatography profiles of purified wild-type CpsUbiX and protein size standards (STD): peak 1, apoferritin (443 kDa); peak 2, *β*-amylase (200 kDa); peak 3, bovine serum albumin (66 kDa); peak 4, carbonic anhydrase (29 kDa). The inset shows the plot of molecular weight *versus* V_e/V_o for protein standards. The elution volume indicated that CpsUbiX was a dodecamer in solution with an apparent molecular weight of 256.3 kDa (V_e = 11.57 ml).

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Table 1
Data-collection statistics.

Values in parentheses are for the last resolution shell.

Data collection	Wild-type (FMN-bound)	V47S mutant (FMN-free)
X-ray source	PAL 7A	PAL 5C
Space group	$C222_1$	$P23$
Unit-cell parameters (Å)	a = 107.2, b = 141.9, c = 170.1	$a = b = c$ = 97.63
Wavelength (Å)	0.97934	0.97952
No. of frames	360	360
Oscillation angle (°)	1	1
Resolution range (Å)	50.0–2.00 (2.03–2.00)	48.81–1.76 (1.86–1.76)
No. of observed reflections	1127237	156791
No. of unique reflections	85707 (4317)	30485 (4430)
Completeness (%)	98.4 (100)	99.1 (99.6)
Multiplicity	13.2 (13.0)	5.1 (5.2)
R_{merge}†	0.120 (0.431)	0.076 (0.183)
Average $I/\sigma(I)$	53.1 (11.9)	14.1 (7.0)

† $R_{merge} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the value of the ith intensity measurement and $\langle I(hkl) \rangle$ is the mean value of all measurements of $I(hkl)$.

2.6. Crystallization and data collection of FMN-bound CpsUbiX

Initial crystallization conditions were searched for using commercially available screening kits, such as the MCSG I–IV series (Microlytic, Burlington, USA), the Wizard Classic I–IV series (Emerald Bio, Seattle, USA) and The Classics and Classics II Suites (Qiagen, Hilden, Germany) screens, and the sitting-drop vapour-diffusion method. The crystallization drops consisted of 0.8 µl protein solution (21.0 mg ml^{-1}) and 0.8 µl crystallization solution. Initial microcrystals were obtained from MCSG III condition No. 94 (0.1 *M* sodium acetate–HCl pH 4.5, 2.5 *M* NaCl, 0.2 *M* lithium sulfate), MCSG IV condition No. 15 (0.1 *M* sodium acetate–HCl pH 4.6, 1.5 *M* lithium sulfate), Wizard II condition No. 8 [0.1 *M* potassium phosphate monobasic/sodium phosphate dibasic pH 6.0, 10%(*w*/*v*) PEG 8000, 0.2 *M* NaCl] and The Classics II Suite No. 61 [0.1 *M* HEPES pH 7.5, 11%(*w*/*v*) PEG 3350, 0.3 *M* L-proline]. These conditions were optimized using variation of the precipitant and pH. In addition, the volume of the drop was increased from 0.8 to 1 µl to generate larger crystals. From these results, the best single crystals of wild-type CpsUbiX were obtained in 200 m*M* sodium chloride, 100 m*M* potassium phosphate monobasic/sodium phosphate dibasic pH 5.8, 11%(*w*/*v*) PEG 8000 at 293 K using the hanging-drop vapour-diffusion method in 24-well plates. The crystal was then immersed and, for cryoprotection, was passed through Paratone-N oil (Hampton Research, Aliso Viejo, USA) for 1 min to remove the remaining aqueous solvent. Finally, the crystal was directly flash-cooled in a nitrogen-gas stream prior to X-ray diffraction data collection. A complete diffraction data set was collected to 2.0 Å resolution in space group $C222_1$, with unit-cell parameters a = 107.2, b = 141.9, c = 170.1 Å (Table 1). Data collection was performed on beamline 7A at the Pohang Accelerator Laboratory (Pohang, Republic of Korea) at a wavelength of 0.97934 Å using an ADSC Quantum 270 CCD detector. During data collection, the distance between the crystal and detector was set to 200 mm and a total of 360 images were collected in 1° oscillation steps with a 3 s exposure per frame. The images were integrated and scaled using *HKL*-2000 (Otwinowski & Minor, 1997).

2.7. Crystallization and data collection of the FMN-free form (V47S mutant) of CpsUbiX

The V47S mutant protein was concentrated to 35.9 mg ml^{-1} by Amicon Ultra-15 centrifugal ultrafiltration (Millipore, Bedford, USA) prior to crystallization. Crystallization of FMN-free CpsUbiX was carried out by the sitting-drop vapour-diffusion technique using commercially available crystallization solutions: the MCSG series (Microlytic, Burlington, USA), the Wizard Classic series (Emerald Bio, Seattle, USA) and The Classics Suite (Qiagen, Hilden, Germany) screens. Equal volumes (0.8 µl) of the protein solution and reservoir solution were mixed and incubated at 293 K in a chamber. Initial small crystals were observed from several conditions: MCSG IV condition No. 15 (0.1 *M* sodium acetate–HCl pH 4.6, 1.5 *M* lithium sulfate), MCSG III condition No. 3 (0.1 *M* Tris–HCl pH 7.0, 1 *M* potassium sodium tartrate, 0.2 *M* lithium sulfate) and The Classics Suite condition No. 57 (0.1 *M* trisodium citrate pH 5.6, 0.5 *M* ammonium sulfate, 1 *M* lithium sulfate). The conditions were optimized by varying the concentration of precipitant and pH to produce suitable crystals for data collection. The best crystals of FMN-free CpsUbiX were obtained using 0.1 *M* trisodium citrate pH 5.4, 0.5 *M* ammonium sulfate, 1.2 *M* lithium sulfate; the drops were prepared by mixing 1 µl protein solution and 1 µl reservoir solution. The crystals grew to maximum dimensions of 0.1 × 0.1 × 0.1 mm after 4 d at room temperature. A single crystal was then soaked and carefully dragged for 1 min in Paratone-N oil (Hampton Research, Aliso Viejo, USA) to remove the mother liquor from the crystal. Prior to X-ray diffraction data collection, the crystal was mounted on a nylon loop and directly flash-cooled in a nitrogen-gas stream at 100 K. A complete data set to 1.76 Å resolution was collected on beamline BL-5C of the Pohang Accelerator Laboratory (Pohang, Republic of Korea). The data were processed with the *HKL*-2000 suite of programs (Otwinowski & Minor, 1997).

3. Results and discussion

3.1. Cloning, expression and purification of wild-type and V47S mutant CpsUbiX

UbiX from *C. psychrerythraea* strain 34H consists of 206 amino-acid residues (calculated molecular weight of 22 552 Da) and has a theoretical isoelectric point of 5.78. Wild-type and V47S mutant CpsUbiX were successfully cloned, overexpressed and purified (Fig. 1*a*). The enzyme purification typically yielded about 20 and 25 mg per litre of wild-type and mutant protein, respectively. The purified wild-type CpsUbiX was yellowish in colour in the absence of FMN, which indicated that sufficient FMN was supplied from the *E. coli* cytosol that was bound tightly to the recombinant CpsUbiX protein (Fig. 1*a*). The wild-type CpsUbiX, under saturated conditions with FMN, exhibited an FMN:protein molar ratio of 1.028:1. On the other hand, the V47S mutant CpsUbiX protein showed an FMN:protein ratio of 0.086:1, suggesting that this single mutation eliminated FMN binding (Figs. 1*b* and 1*c*). The CD data suggested that the mutant protein had a similar secondary structure compared with the wild-type protein, which indicates that the mutation resulted in FMN-free protein, but it did not affect overall protein folding (Fig. 1*d*). According to our analytical size-exclusion chromatography data, the purified wild-type eluted as a single peak with a corresponding molecular weight of approximately 256.3 kDa, indicating that CpsUbiX is a dodecamer in solution (Fig. 1*e*).

3.2. Crystallographic studies

The best crystals of wild-type CpsUbiX formed within 3 d using 200 m*M* sodium chloride, 100 m*M* potassium phosphate monobasic/sodium phosphate dibasic pH 5.8, 11%(*w*/*v*) PEG 8000. The wild-type CpsUbiX crystal (Fig. 2*a*) belonged to space group $C222_1$ and had unit-cell parameters a = 107.2, b = 141.9, c = 170.1 Å. A diffraction

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image of the wild-type CpsUbiX crystal is shown in Fig. 2(*c*). Assuming the presence of a hexamer in the asymmetric unit, the Matthews coefficient (Matthews, 1968) was calculated to be 2.39 Å^3 Da^{-1}, which corresponds to a solvent content of 48.57%. The initial model of wild-type CpsUbiX was obtained by the molecular-replacement method using *Phaser* (McCoy *et al.*, 2007). The coordinates of monomeric UbiX from *P. aeruginosa* (PDB entry 3zqu, Kopec *et al.*, 2011; sequence identity 65%) were used as a search model. The initial *R* value after molecular replacement was 45.6%, with a rotation-function *Z*-score of 4.0 and a translation-function *Z*-score of 29.1. The resulting PDB file was refined against the original data set using the macromolecular crystallographic refinement program *REFMAC*5 (Murshudov *et al.*, 2011), and had reasonable R_{work} and R_{free} values of 16.8 and 22.4%, respectively. After several rigid-body and restrained refinement steps, the resultant electron-density map was sufficient to build most residues and bound FMN molecules. The wild-type CpsUbiX crystal (space group $C222_1$) contained six subunits in the asymmetric unit. However, application of the crystallographic symmetry operator generated a compact dodecamer structure, which is consistent with the results of analytical size-exclusion chromatography. Further studies on structure refinement and model building of wild-type CpsUbiX are currently under way.

In this study, the Val47 residue was mutated to serine to eliminate the hydrophobic interaction with FMN. The V47S mutation resulted in a complete loss of FMN binding and allowed us to produce and crystallize FMN-free CpsUbiX protein. The best crystallization condition for the V47S mutant CpsUbiX was in a reservoir solution consisting of 0.1 *M* trisodium citrate pH 5.4, 0.5 *M* ammonium sulfate, 1.2 *M* lithium sulfate (Fig. 2*b*). The crystal diffracted to 1.76 Å resolution and belonged to the primitive cubic space group *P*23, with unit-cell parameters $a = b = c = 97.6$ Å (Fig. 2*d*). The calculated Matthews coefficient value of 3.44 Å^3 Da^{-1} indicated the presence of a monomer in the asymmetric unit, with 64.23% solvent content. This

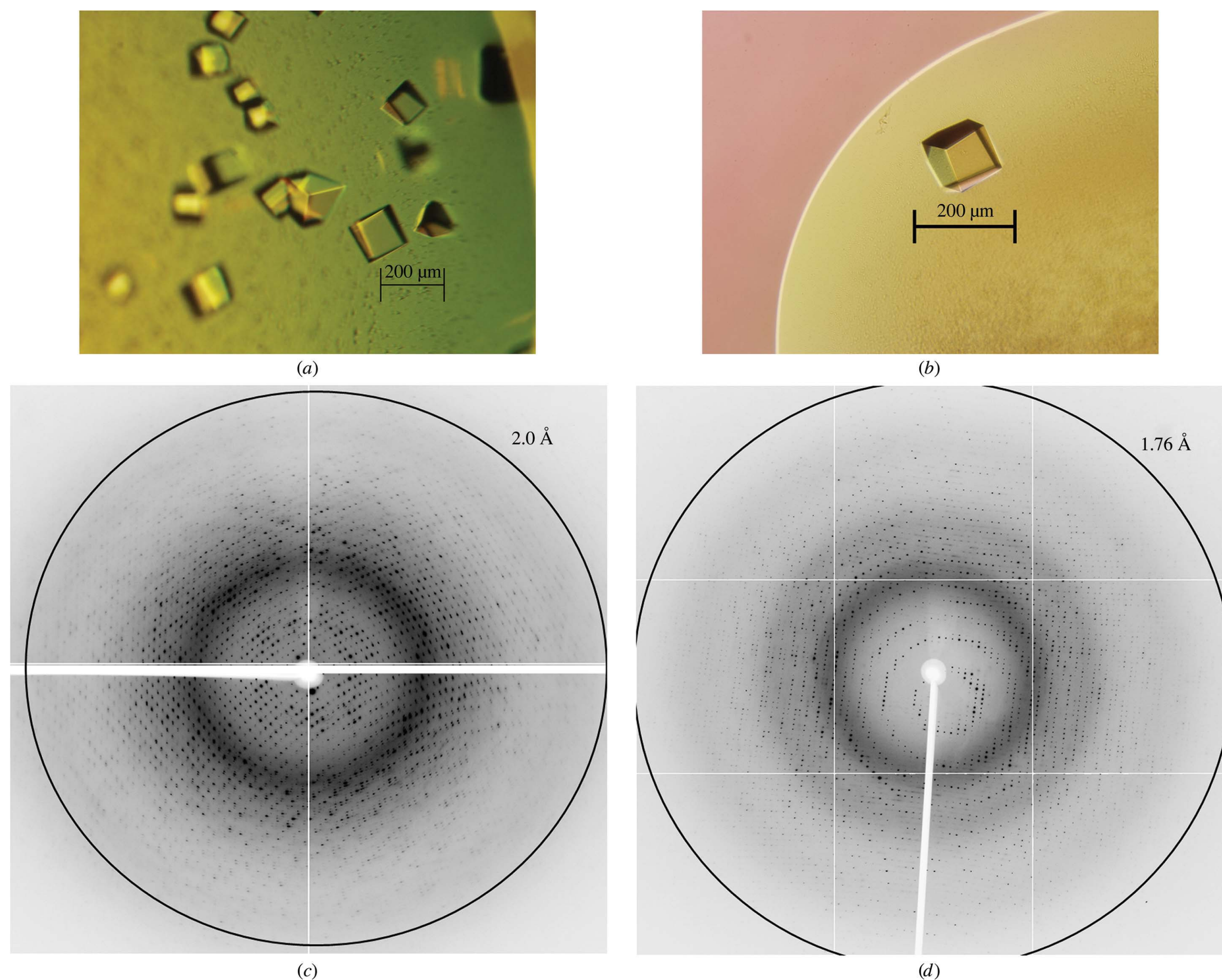

Figure 2
Crystal and X-ray diffraction images of the wild type and V47S mutant of CpsUbiX. (*a*) A crystal of wild-type CpsUbiX obtained using 200 m*M* sodium chloride, 100 m*M* potassium phosphate monobasic/sodium phosphate dibasic pH 5.8, 11%(*w*/*v*) PEG 8000. Its approximate dimensions were 0.1 × 0.15 × 0.15 mm. (*b*) A crystal of V47S mutant CpsUbiX obtained using 0.1 *M* trisodium citrate pH 5.4, 0.5 *M* ammonium sulfate, 1.2 *M* lithium sulfate. Its approximate dimensions were 0.1 × 0.1 × 0.1 mm. (*c*) X-ray diffraction image (2.0 Å resolution) from a crystal of wild-type CpsUbiX. (*d*) X-ray diffraction image (1.76 Å resolution) of the V47S mutant CpsUbiX crystal obtained during data collection.

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initial structure was solved by molecular replacement with *MOLREP* (Vagin & Teplyakov, 2010), using the FMN-free wild-type CpsUbiX structure as a search model. The resulting PDB file was refined against the original data set using the macromolecular crystallographic refinement program *REFMAC*5 (Murshudov *et al.*, 2011), and had reasonable R_{work} and R_{free} values of 25.9 and 29.0%, respectively. The crystallographic asymmetric unit of the V47S mutant structure contained a monomer. However, application of the crystallographic symmetry operator generated the same dodecamer structure as found for the wild-type CpsUbiX crystal, which is in agreement with our analytical size-exclusion chromatography data. The CpsUbiX dodecamer structures generated *via* the crystallographic symmetry operators exhibited close structural homology to the previously determined dodecamer structures of *P. aeruginosa* UbiX (Kopec *et al.*, 2011) and *E. coli* Pad1 (Rangarajan *et al.*, 2004). These observations strongly suggest that the CpsUbiX protein is also biologically active in its dodecamer form.

In conclusion, we successfully overexpressed, purified and crystallized FMN-bound and FMN-free forms of CpsUbiX. Currently, model building, structural refinement and further biochemical studies are ongoing. A more detailed structural comparison using fully refined structure models will contribute to a better understanding of the molecular mechanisms of this important enzyme.

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