Crystallization and preliminary X-ray analysis of the hypothetical deaminase RPB_0146 from *Rhodopseudomonas palustris* HaA2

Guofang Zhang,^{a,b} Dan Yu,^{a,b}
Guodong Yang,^{b,c} Hui Dong,^b
Tongcun Zhang^a and Xiang Liu^{b*}

^aTianjin University of Science and Technology, Tianjin 300222, People's Republic of China, ^bTianjin International Joint Academy of Biotechnology and Medicine, Tianjin 300457, People's Republic of China, and ^cNankai University, Tianjin 300071, People's Republic of China

Correspondence e-mail: xiang.liu@htmdc.org

Received 12 May 2014

Accepted 12 August 2014

RPB_0146, a putative deaminase from *Rhodopseudomonas palustris* HaA2, was expressed in *Escherichia coli* BL21 (DE3) cells and purified using a His₆ tag by Ni²⁺-chelating affinity chromatography for X-ray crystallographic analysis. Diffraction-quality crystals were grown by the hanging-drop vapour-diffusion method at 289 K and diffracted to a resolution of 2.44 Å using a wavelength of 1.000 Å at the Photon Factory (KEK), Japan. The crystals belonged to the orthorhombic space group *P*₂₁₂₁₂₁, with unit-cell parameters *a* = 66.26, *b* = 123.94, *c* = 155.95 Å.

1. Introduction

Chlorinated nitroaromatic compounds (CNAs), including chloronitrobenzenes, chloronitrophenols and chloronitrobenzoic acids, which are introduced into the environment mainly by improper waste disposal in agriculture and industry, are very toxic to human health (Yong & Zhong, 2010). Microorganisms have been widely studied for the biodegradation of CNAs because of their low emission into the environment and the few byproducts formed during their function (Arora *et al.*, 2012). In order to solve the environmental pollution problems caused by CNAs in China, Chinese scientists have made considerable progress in using microorganisms or enzymes to break down the toxic CNAs (Yong & Zhong, 2010). For example, the biodegradation pathway of 4-chloronitrobenzene (4-CNB), as a model compound for research on the CNAs, has been extensively studied in China in recent years (Liu *et al.*, 2007; Wu *et al.*, 2005, 2006; Ma *et al.*, 2007). In this pathway, a new type of deaminase has been identified. The new deaminases use 2-amino-5-chloromuconate, an important intermediate in 4-CNB degradation, as the substrate. These deaminases have no known cofactor-binding sites and no significant sequence similarity to other known deaminases. However, they show sequence similarity to a conserved domain of glycosyl hydrolases, the function of which has not been shown to be related to deaminase activity (Liu *et al.*, 2007). To date, no structural information has been obtained to explain these unique features.

RPB_0146 from *Rhodopseudomonas palustris* HaA2 is a member of this new deaminase family (Liu *et al.*, 2007). RPB_0146 is encoded by ABD04858 from *R. palustris* HaA2, a bacterium that has a strong biodegradation capability on aromatic compounds and is largely used to remediate pollution caused by industrial waste (Oda *et al.*, 2004; Okubo *et al.*, 2006; Van der Woude *et al.*, 1994). The study of the crystal structure of RPB_0146 could potentially elaborate and explain the new features of this new deaminase family. Here, we describe the cloning, expression, purification, crystallization and preliminary X-ray diffraction studies of the RPB_0146 protein from *R. palustris* HaA2.

2. Materials and methods

2.1. Cloning, expression and purification

The gene encoding the RPB_0146 protein (GenBank accession No. ABD04858) was optimized and chemically synthesized for expression in *Escherichia coli* based on the released sequence in GenBank.

The gene was amplified by PCR using the forward primer 5'-CTA-CATATGATCGAGACCGTCGAAGCCCCG-3' and the reverse primer 5'-AGACTCGAGGACCACGCGCTCGCGA-3'. The *NdeI* and *XhoI* restriction sites are underlined. The PCR product was digested with *XhoI* and *NdeI* and subsequently inserted into the pET-22b(+) plasmid (Novagen, USA), which encodes a C-terminal His₆ tag. All plasmids were verified by sequencing (GENEWIZ, Beijing, People's Republic of China). The recombinant plasmid was transformed into *E. coli* BL21 (DE3) competent cells (Novagen, USA). The transformants were grown at 310 K in Luria-Bertani (LB) medium containing 100 µg ml⁻¹ ampicillin until the OD₆₀₀ reached 0.6–0.8 and were induced overnight with 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG).

The cells were harvested by centrifugation at 8600g for 30 min, resuspended in 20 ml lysis buffer (20 mM Tris-HCl pH 7.5, 200 mM NaCl, 10 mM imidazole) and then lysed using a high-pressure homogenizer (JNBio, People's Republic of China) on ice. The cell lysate was clarified by centrifugation for 30 min at 48 400g and 277 K and the supernatant was loaded onto an Ni²⁺-chelating affinity-chromatography column. The column was rinsed with 20 ml washing buffer (20 mM Tris-HCl pH 7.5, 200 mM NaCl, 20 mM imidazole) to remove unbound proteins. RPB_0146 was eluted with 20 ml elution buffer (20 mM Tris-HCl pH 7.5, 200 mM NaCl, 200 mM imidazole). Subsequent purification was performed by anion-exchange chromatography on a Resource Q column (GE Healthcare, USA). The protein was pooled, concentrated and dialyzed against 20 mM Tris-HCl buffer pH 7.5 by ultrafiltration with an Amicon Ultra-15 centrifugal filter device (10 000 Da molecular-weight cutoff, Millipore). The purity of the protein was analysed by 12% SDS-PAGE (Fig. 1) and the concentration was determined using a NanoDrop device at 280 nm (Thermo Scientific, USA). Macromolecule-production information is summarized in Table 1.

2.2. Crystallization

Crystallization experiments were performed at 289 K using the hanging-drop vapour-diffusion method with Crystal Screen, Crystal Screen 2, Index, SaltRx and PEG/Ion from Hampton Research. The protein concentrations, which were calculated by the NanoDrop device and divided by the extinction coefficient, were between 10 and

Figure 1
12% SDS-PAGE analysis of RPB_0146. Lane M, molecular-mass standards (labelled in kDa); lane 1, purified RPB_0146 protein (29 kDa).

Table 1
Macromolecule-production information.

Source organism	<i>R. palustris</i> HaA2
DNA source	Chemical synthesis
Forward primer	5'-CTACATATGATCGAGACCGTCGAAGCCCCG-3'
Reverse primer	5'-AGACTCGAGGACCACGCGCTCGCGA-3'
Cloning vector	pET-22b(+) plasmid
Expression vector	pET-22b(+) plasmid
Expression host	<i>E. coli</i> BL21 (DE3)
Complete amino-acid sequence of the construct produced	MIETVEAPNSGYRYMPGVFQYSCGIAALPGFAIERVRFAEPV-PLKQGFAKIAEILKAAGRPLTAFGACELRSPAPFTEDGFK-AFNEIYIQTLDVWGIMDKGINPIARSNVCPQIDPPAEPSEYAFSYTVPADNAPKSFVIAGSGEAPKGNRYRDHTVALGDTSPAGLQKKAQFVLGEMERRMSAFGGSWRDITGAQLYTVH-DIHPFLESELGNRGVFRHGLTWHFNRPPVEGLDYEMDCRCVHRRERVVLEHHHHHH

Table 2
Crystallization.

Method	Hanging-drop vapour diffusion
Plate type	16-well hanging-drop plate
Temperature (K)	289
Protein concentration (mg ml ⁻¹)	10–15
Extinction coefficient (ml mg ⁻¹ cm ⁻¹)	1.048
Buffer composition of protein solution	20 mM Tris-HCl pH 7.5
Composition of reservoir solution	0.2 M bis-tris pH 7.0, 2.2 M ammonium sulfate, 4% (w/v) PEG 400
Volume and ratio of drop	1 µl protein solution:1 µl reservoir solution
Volume of reservoir (µl)	300

Table 3
Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	BL-5A, Photon Factory
Wavelength (Å)	1.000
Temperature (K)	100
Detector	ADSC Q315
Crystal-to-detector distance (mm)	369.40
Rotation range per image (°)	0.5
Total rotation range (°)	360
Exposure time per image (s)	1
Space group	<i>P</i> ₂ ₁ ₂ ₁
<i>a</i> , <i>b</i> , <i>c</i> (Å)	66.18, 124.03, 157.31
Mosaicity (°)	0.67
Resolution range (Å)	50.00–2.44 (2.48–2.44)
Total No. of reflections	693173 (25450)
No. of unique reflections	48815 (2383)
Completeness (%)	99.9 (99.5)
Multiplicity	14.2 (10.7)
Average <i>I</i> /σ(<i>I</i>)	69.4 (9.5)
<i>R</i> _{merge} † (%)	10.8 (38.3)
Overall <i>B</i> factor from Wilson plot (Å ²)	44.10

† $R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$, where $I_i(hkl)$ is the intensity of the *i*th measurement of reflection *hkl* and $\langle I(hkl) \rangle$ is the mean intensity of all symmetry-related reflections.

15 mg ml⁻¹. Drops were prepared by mixing 1 µl purified protein solution and 1 µl reservoir solution and were equilibrated against 300 µl reservoir solution. Crystallization conditions were optimized based on the initial screenings. Crystallization information is summarized in Table 2.

2.3. Data collection and processing

The diffraction data were collected on beamline BL-5A at the Photon Factory (KEK), Japan. For X-ray diffraction experiments, the crystals were quick-soaked in reservoir solution containing 20% (v/v) glycerol as a cryoprotectant, flash-cooled in liquid nitrogen (Parkin & Hope, 1998) and maintained on the goniometer at 100 K in a stream of cold nitrogen. Diffraction data were collected from a single crystal at a wavelength of 1.000 Å: 720 frames were collected with 0.5°

Figure 2
Typical crystals of RPB_0146 (about $0.1 \times 0.5 \times 1.0$ mm in size).

Figure 3
Diffraction pattern of an RPB_0146 crystal.

oscillation per image. The data were indexed, integrated and scaled by *HKL-2000* (Otwinowski & Minor, 1997). Data-collection statistics are summarized in Table 3.

3. Results and discussion

The RPB_0146 gene was cloned into the pET-22b(+) vector and expressed in *E. coli* BL21 (DE3) cells. The recombinant protein

RPB_0146 with a C-terminal His₆ tag was successfully expressed and purified to electrophoretic homogeneity. The molecular mass of RPB_0146 was estimated to be about 30 kDa by SDS-PAGE (Fig. 1), coinciding with the predicted molecular mass of 29 kDa of recombinant RPB_0146 with a His₆ tag at the C-terminus.

Initial laminar crystals of RPB_0146 protein were obtained in Index condition No. 68 after initial sparse-matrix screening, but failed to diffract. Following further optimization, thick crystals were obtained with mother liquor consisting of 0.2 M bis-tris pH 7.0, 2.2 M ammonium sulfate, 4% (w/v) PEG 400. The crystal dimensions were about $0.1 \times 0.5 \times 1.0$ mm (Fig. 2).

A diffraction data set was collected to 2.44 Å resolution from a native RPB_0146 crystal (Fig. 3) with an R_{merge} of 10.8%. The crystals belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 66.18$, $b = 124.03$, $c = 157.31$ Å. Assuming the presence of four molecules per asymmetric unit, the Matthews coefficient was $2.78 \text{ Å}^3 \text{ Da}^{-1}$, corresponding to a solvent content of 55.83% (Matthews, 1968).

The RPB_0146 protein is a conserved hypothetical deaminase. To date, there are no models with a sequence homology of greater than 30% in the Protein Data Bank (Bernstein *et al.*, 1977); therefore, the molecular-replacement method (MR) cannot be used for structure determination. We have therefore labelled the protein with selenomethionine in attempt to obtain the initial phase. We hope that the RPB_0146 protein structure will provide insight into the biochemical properties of this new type of deaminase in biodegradation.

This work was supported by grants from the National Natural Science Foundation of China (31170116, 31200641 and 81102374).

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