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Crystallization and preliminary X-ray crystallographic analysis of PhoK, an extracellular alkaline phosphatase from *Sphingomonas* sp. BSAR-1

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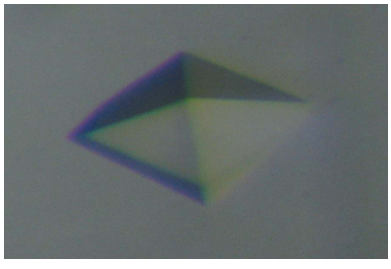

Alkaline phosphatases (APs) are widely distributed from microbes to humans and are involved in several important biological processes such as phosphate nutrition, signal transduction and pathogenesis. Alkaline phosphatases are also useful in various industrial applications and in recombinant DNA technology. A new AP enzyme from *Sphingomonas* sp. strain BSAR-1, termed PhoK, has been shown to be useful in uranium bioprecipitation. PhoK was expressed, purified and crystallized. The crystals belonged to space group $P4_32_12$ or $P4_12_12$, with unit-cell parameters $a = b = 87.37$, $c = 168.16$ Å, and contained one enzyme molecule in the asymmetric unit. Native diffraction data have been collected to 1.95 Å resolution at the ESRF.

1. Introduction

Phosphates are essential for living organisms, which contain orthophosphates, pyrophosphates, polyphosphates, nucleotides, sugar phosphates and phosphorylated derivatives of organic compounds. Phosphatases play a crucial role in supporting microbial nutrition by releasing assimilable phosphate from various organic sources. The alkaline phosphatases constitute a superfamily of metalloenzymes that includes phosphatases, phosphodiesterases and sulfatases among others (Zalatan *et al.*, 2006; Galperin *et al.*, 1998). Alkaline phosphatases (EC 3.1.3.1), hereafter referred to as APs, are enzymes that hydrolyze phosphate monoesters to a phosphate ion and an alcohol at alkaline pH. The hydrolysis of phosphate monoesters is an important biological process that is involved in various metabolic and cellular signal-transduction pathways. APs are highly conserved from prokaryotes to eukaryotes (O'Brien & Herschlag, 2001).

The functions of APs are still not fully understood. In microbes they are involved in phosphorus nutrition, especially during phosphate starvation (Hou *et al.*, 1966). Most APs are induced by free inorganic phosphate limitation, although constitutive AP activity has also been observed in bacteria located in the rumen (Forsberg & Cheng, 1980). Microbial APs also play indispensable roles in signal transduction (Hulett, 1996) and the virulence of bacteria (Kadurugamuwa & Beveridge, 1997). The availability of molecular sequence data has led to the identification of molecular families of phosphohydrolases for which signature sequence motifs have been defined (Thaller *et al.*, 1998). A great divergence of enzyme characteristics has also been observed within these families (Berlutti *et al.*, 2001; Goldman *et al.*, 1990; Gomez & Ingram, 1995; Wagner *et al.*, 1995).

The catalytic reaction of APs with various synthetic chromogenic substrates has led to their usage in immunodetection techniques such as ELISA and Western blotting (Tomazic-Allen, 1991). They are also used in recombinant DNA technology. We recently reported an AP, termed PhoK, from *Sphingomonas* sp. strain BSAR-1 (Nilgiriwala *et al.*, 2008). PhoK was found to exhibit several unique features, such as constitutive expression, extracellular release, a very large molecular size of the active protein (~200 kDa) and high specific activity. We also reported the successful application of a recombinant *Escherichia coli* strain overexpressing PhoK for the bioprecipitation of uranium

as uranyl phosphate under alkaline conditions (Nilgiriwala *et al.*, 2008). Considering the possible potential of PhoK in various applications, it was considered desirable to understand the structure–function relationships of this protein. With this aim, the crystallization of PhoK was initiated. In this communication, we report the crystallization and preliminary X-ray crystallographic analysis of purified recombinant PhoK enzyme from *Sphingomonas* sp. strain BSAR-1.

2. Materials and methods

2.1. Overexpression and purification of PhoK protein

The cloning of the *phoK* gene from *Sphingomonas* sp. BSAR-1 in *E. coli* BL21 (DE3) pLysS has been reported previously (Nilgiriwala *et al.*, 2008). The PhoK-overexpressing recombinant *E. coli* strain, denoted EK4, was grown aerobically at 310 K in Luria–Bertani (LB) medium containing 33 µg ml^{-1} chloramphenicol and 50 µg ml^{-1} kanamycin until the culture attained an $OD_{600\,nm}$ of 0.8. The cells were subsequently induced by 1 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) at 303 K for 4 h under agitation at 180 rev min^{-1}. The recombinant (His_6-tagged) PhoK-overexpressing cells were lysed by sonication (Branson, Germany) in 100 m*M* Tris, 100 m*M* NaCl buffer containing 0.5 *M* urea. The enzyme was purified from the soluble cell-free lysate by Ni^{2+}–nitrilotriacetic acid (Ni^{2+}–NTA) affinity chromatography. All purification steps were carried out at 277 K and as per QIAexpressionist protocols (Qiagen, USA).

The purified recombinant protein was dialyzed against 50 m*M* Tris, 100 m*M* NaCl buffer at 277 K. The protein was then concentrated using a Centricon centrifugal concentrator (10 000 Da molecular-mass cutoff). The purity of the protein was determined after resolving it by SDS–PAGE on 10% resolving gel with Coomassie Brilliant Blue staining. The concentrated PhoK protein was used for crystallization without removing the His tag.

2.2. Crystallization of PhoK

Screening for crystallization conditions was performed by the sitting-drop vapour-diffusion method in 96-well crystallization plates (Greiner) using a CyBio HTPC robot operated at 298 K. Drops were prepared by mixing protein solution (3–5 mg ml^{-1}) with an equal volume of reservoir solution and were equilibrated against 75 µl reservoir solution. Initial screening was performed using commercially available crystallization screens (Structure Screens I and II from Molecular Dimensions Ltd and Wizard I and II from Emerald BioSystems).

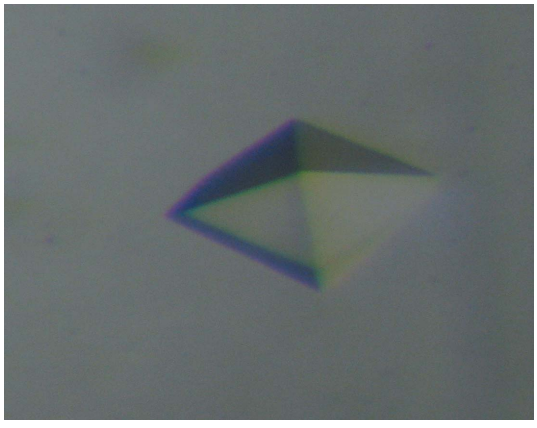

Figure 1
Single crystal of PhoK from *Sphingomonas* sp. BSAR-1 in a crystallization drop of total volume 1.8 µl.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

X-ray source	FIP-BM30A beamline, ESRF
Wavelength (Å)	0.976180
Temperature (K)	100
Resolution range (Å)	50–1.95 (2.05–1.95)
Space group	$P4_32_12$ or $P4_12_12$
Unit-cell parameters (Å)	a = 87.37, b = 87.37, c = 168.16
No. of reflections measured	365046
No. of unique reflections	82621
No. of molecules in ASU	1
Completeness (%)	91.1 (78.6)
$I/\sigma(I)$	13.75 (2.61)
$R_{mrgd\text{-}F}$† (%)	9.9 (52.7)
Mosaicity (°)	0.155

† Diederichs & Karplus (1997).

2.3. X-ray diffraction data collection

For X-ray diffraction data collection, crystals were soaked in mother liquor containing 25% glycerol for 10–20 s and flash-cooled in liquid nitrogen. X-ray diffraction data were collected at 100 K on the FIP-BM30A beamline at the ESRF (Roth *et al.*, 2002) using the oscillation method. A total of 110 frames were collected at a crystal-to-detector distance of 269.10 mm with 1.0° oscillation per frame and an exposure time of 30 s. The diffraction data were processed using the *XDS* software suite (Kabsch, 1993).

3. Results and discussion

Crystals of the purified protein appeared under several conditions from the crystallization screens. Crystals grown in these screens were used directly for diffraction studies. The present analysis was conducted using a crystal grown in the condition 1.6 *M* ammonium sulfate, 10%(*v*/*v*) dioxane, 100 m*M* MES pH 6.5 (Fig. 1). The crystal used for diffraction data collection was approximately 0.1 × 0.07 × 0.07 mm in size. The crystal belonged to space group $P4_32_12$ or $P4_12_12$, with unit-cell parameters $a = b = 87.37$, $c = 168.16$ Å. The calculated V_M value (Matthews, 1968) of 2.55 Å^3 Da^{-1} suggested the presence of one protein molecule per asymmetric unit, with a solvent content of 51.82%. The crystal diffracted to 1.95 Å resolution at 100 K. Preparation of a selenomethionine derivative is in progress. Structure solution will be attempted using the molecular-replacement and MAD/SAD methods.

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