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Purification, crystallization and preliminary X-ray studies of thermostable alkaline phosphatase from *Thermus* sp. 3041

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Thermostable alkaline phosphatase from *Thermus* sp. 3041 has been expressed in *Escherichia coli*, purified and crystallized. The crystals belong to space group $P2_122_1$, with unit-cell parameters a = 57.7, b = 69.9, c = 111.5 Å. Diffraction data were collected to 2.54 Å with a completeness of 91.1% (87.8% for the last shell), an $R_{\rm merge}$ value of 0.105 (0.312) and an $I/\sigma(I)$ value of 9.5 (3.6).

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1. Introduction

Why do some organisms survive temperatures above 373 K while others cease to live at 313 K? Why are some proteins extremely thermostable while others denature at low temperature? Though such queries have prompted theoretical and experimental research for many years, the phenomenon has been only partially understood despite its great importance in both the scientific and industrial arenas (Vogt *et al.*, 1997). Site-directed mutagenesis and comparison of the amino-acid sequences and tertiary structures of homologous proteins from thermophiles and mesophiles has been widely performed in order to study the mechanism of protein thermostability (Vogt *et al.*, 1997; Querol *et al.*, 1996; Korolev *et al.*, 1995; Erduran & Kocabiyik, 1998). Random mutagenesis might be a more efficient way to study the mechanism of protein thermostability, as all the mutations are responsible for thermostability with no subjectivity.

Alkaline phosphatase (AP; E.C. 3.1.3.1), a non-specific monoesterase, belongs to the class of enzymes that catalyze the displacement reaction at phosphorus (Knowles, 1980). Thermostable alkaline phosphatase from *Thermus* sp. 3041 (TAP) is an AP with a high thermostability and a high stability in high concentrations of detergent. The enzyme retains 75% of its activity after incubation at 368 K for 60 min (Sheng *et al.*, 1997). The gene encoding TAP has been cloned in *E. coli* (Yuan *et al.*, 1998). The amino-acid sequence homology between TAP and the mesophilic AP from *E. coli* is 27.5%. As an AP, TAP produces inorganic phosphate and a yellow-coloured alcohol when using the colorless *p*-nitrophenyl phosphate as a substrate. It is easy to screen the mutants with altered thermostability from random mutagenesis by observing *in situ* colony coloration after temperature treatment. 40 mutants with changed thermostability have been obtained by PCR-mediated random mutagenesis; the screening of further mutants and DNA sequencing are currently under way. The crystal structure will help to understand the mechanism of the high thermostability of TAP and the reasons why the mutants possess altered thermostability.

2. Material and methods

2.1. Protein purification

The gene encoding thermostable alkaline phosphatase was cloned into pJLA503 (Schauder *et al.*, 1987) and overexpressed in *E. coli* TG1. Approximately 10 g (wet weight) of cells were disrupted by sonication in 100 ml buffer *A* containing 50 m*M* Tris–HCl pH 7.0, 2 m*M* EDTA, 10 m*M* β-mercaptoethanol. The lysate was incubated at 348 K for 30 min and then cooled on ice. The cell debris and denatured proteins were removed by centrifugation and the supernatant was applied to Phenyl Sepharose 6 Fast Flow (Amersham Pharmacia) equilibrated with buffer *A*. The column was eluted with a pH gradient of buffer *A* to 10 m*M* Tris–HCl pH 12.0 at 298 K. The purified TAP showed a single band on SDS–PAGE and was concentrated to 10 mg $\rm ml^{-1}$ in 10 m*M* Tris–HCl buffer pH 8.0 at 298 K.

2.2. Crystallization and preliminary X-ray analysis

Crystallization was performed at 277 K using the hanging-drop vapour-diffusion method with equal volumes of protein and precipitant solution (5 µl). The Crystal Screen Lite kit (Hampton Research) was used to screen the crystallization conditions.

X-ray intensity data were collected on a MAR Research 300 mm image plate at 298 K. Cu $K\alpha$ X-rays were used and the generator was set at 40 kV and 50 mA. The distance to the

Table 1
Crystal parameters and data-collection statistics for TAP.

Values in parentheses are for the highest resolution shell.

Space group	$P2_122_1$
Unit-cell parameters (Å)	a = 57.7, b = 69.9, c = 111.5
V_M (Å^3 Da^{-1})	2.16
Resolution (Å)	100–2.54 (2.64–2.54)
No. of observations	248128
No. of unique reflections	22992
R_{merge} (%)	10.5 (31.2)
Completeness (%)	91.1 (87.8)
$I/\sigma(I)$	9.5 (3.6)

image plate was set to 175 mm and the images were recorded with 1° oscillation per image and an exposure time of 600 s per frame. Two data sets with 90 images each were collected. The data were autoindexed, integrated and corrected for Lorentz and polarization effects using *DENZO* on a Silicon Graphics INDY system. Data were scaled and merged using the program *SCALEPACK* (Otwinowski, 1993).

3. Conclusions

Small crystals were observed in several drops from the Crystal Screen Lite kit. The initial conditions were optimized and the crystals used for data collection were grown at 277 K by combining 5 µl of 10 mg ml^{-1} protein solution and 5 µl of 140 m*M* $MgCl_2$, 100 m*M* HEPES buffer pH 7.5 at 298 K, 28.5%(*w*/*v*) polyethylene glycol 400. The crystals grew to maximum dimensions of 0.8 × 0.5 × 0.1 mm (Fig. 1).

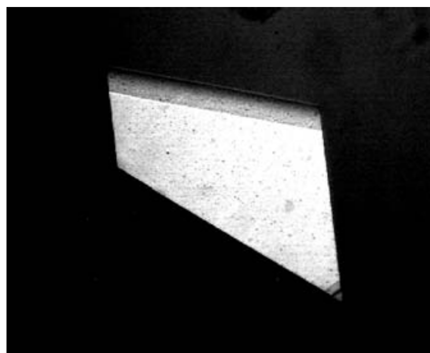

Figure 1
Crystal of thermostable alkaline phosphatase from *Thermus* sp. 3041. The dimensions of the largest crystal were 0.8 × 0.5 × 0.1 mm.

TAP crystals belong to the orthorhombic space group $P2_122_1$, with unit-cell parameters a = 57.7, b = 69.9, c = 111.5 Å. A calculation assuming one 52 kDa subunit per asymmetric unit yields a V_M of 2.16 Å^3 Da^{-1} and a solvent content of 43% (Matthews, 1968). The data statistics are given in Table 1. A search for heavy-atom derivatives for structure solution by multiple isomorphous replacement is currently under way.

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