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Received 2 November 2005

Accepted 11 December 2005

Crystallization and preliminary crystallographic analysis of human common-type acylphosphatase

Human acylphosphatase, an 11 kDa enzyme that catalyzes the hydrolysis of carboxyl phosphate bonds, has been studied extensively as a model system for amyloid-fibril formation. However, the structure is still not known of any isoform of human acylphosphatase. Here, the crystallization and preliminary X-ray diffraction data analysis of human common-type acylphosphatase are reported. Crystals of human common-type acylphosphatase have been grown by the sitting-drop vapour-diffusion method at 289 K using polyethylene glycol 4000 as precipitant. Diffraction data were collected to 1.45 Å resolution at 100 K. The crystals belong to space group $P2_12_12_1$, with unit-cell parameters $a = 42.58$, $b = 47.23$, $c = 57.26$ Å.

1. Introduction

Acylphosphatase (EC 3.6.1.7) is a small cytoplasmic enzyme (~10 kDa) that is widely distributed in all three domains of living organisms: eukarya, bacteria and archaea. It catalyses the hydrolysis of the carboxyl phosphate bonds of acylphosphates such as 1,3-bisphosphoglycerate, carbamoylphosphate and succinylphosphate, which are essential intermediates in glycolysis, the TCA cycle, pyrimidine biosynthesis and the urea cycle (Stefani *et al.*, 1997). Mammalian acylphosphatases are classified into two isoforms, the muscle and common types, which share more than 50% sequence identity. The biological role of acylphosphatase is not fully understood. The enzyme hydrolyzes 1,3-bisphosphoglycerate to 3-phosphoglycerate and uncouples the ATP-generating reaction catalyzed by phosphoglycerate kinase (Harary, 1957; Ramponi *et al.*, 1988; Raugei *et al.*, 1996; Dan'shina *et al.*, 2003). Its role in increasing the rate of glycolysis is further supported by the fact that the enzyme is overexpressed in tissues (*e.g.* brain, heart, skeletal muscle) that are active in glycolysis (Stefani *et al.*, 1997). Acylphosphatase can hydrolyze the β -aspartyl phosphate intermediate of Ca^{2+} - and Na^+, K^+ -ATPase and may play a role in regulating ion transport across membranes (Nassi *et al.*, 1991; Nediani *et al.*, 1995, 1996, 1999).

Human acylphosphatases have been studied extensively as a model for the formation of insoluble protein aggregates known as amyloid fibrils (Taddei *et al.*, 2000, 2001; Chiti *et al.*, 1999, 2000, 2001; Chiti, Calamai *et al.*, 2002; Chiti, Taddei *et al.*, 2002). Mutations that destabilize the native state of this enzyme have been shown to promote aggregation by populating the partially unfolded intermediates that serve as a precursor to insoluble aggregates. These studies indicate that aggregation rate correlates well with simple biophysical properties of human acylphosphatase such as hydrophobicity, secondary-structure propensity and net charges (Chiti *et al.*, 2003).

The structures of acylphosphatases from various species have been determined. The structure of horse muscle-type acylphosphatase has been determined by NMR spectroscopy (Pastore *et al.*, 1992) and the structures of common-type acylphosphatase from bovine testis (Thunnissen *et al.*, 1997) and acylphosphatases from *Drosophila* (Zuccotti *et al.*, 2004) and from the hyperthermophilic archaeon *Pyrococcus horikoshii* (Cheung *et al.*, 2004, 2005) have been determined by X-ray crystallography. Recently, preliminary crystallographic data have been reported for an archaeal acylphosphatase from *Sulfolobus solfataricus* (Zuccotti *et al.*, 2005). To our knowledge,

Table 1

Data-processing statistics for human common-type acylphosphatase.

Values in parentheses are for the highest resolution shell.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	$a = 42.58, b = 47.23, c = 57.26$
Resolution (Å)	17.70–1.45 (1.50–1.45)
No. of measurements	89366
No. of unique reflections	20888
Redundancy	4.28 (2.97)
Completeness (%)	99.1 (95.4)
$R_{\text{merge}}^{\dagger}$ (%)	3.2 (28.9)
Mean $I/\sigma(I)$	19.8 (2.9)

$$^{\dagger} R_{\text{merge}} = \sum_{hkl} |I - \langle I \rangle| / \sum_{hkl} I.$$

no structure of human acylphosphatase is available, although it has been widely studied as a paradigm for amyloid-fibril formation. Here, we report the crystallization and preliminary X-ray diffraction data analysis of human common-type acylphosphatase.

2. Expression and purification

The sequence encoding the human common-type acylphosphatase (11.3 kDa) was amplified by polymerase chain reaction from human liver cDNA. The amplified fragment was subcloned into pET507a, an in-house modified vector with a multiple cloning site inserted between the *Nco*I and *Bam*HI sites of pET3d (Novagen). *Escherichia coli* C41 cells (Miroux & Walker, 1996) transformed with the expression plasmid were grown in M9ZB medium at 310 K until the absorbance at 600 nm reached 0.4–0.6, when isopropyl- β -thiogalactopyranose was added to a concentration of 0.2 mM to induce protein expression. After incubation at 310 K for 16–20 h, cells were harvested by centrifugation and resuspended in 20 mM Tris pH 7.5 with 0.5 mM phenylmethylsulfonyl fluoride and lysed by sonication. The lysed cells were centrifuged at 13 000g for 30 min. The supernatant was applied onto a HiTrap SP HP column (Amersham Biosciences) equilibrated with 20 mM Tris buffer pH 7.5 (buffer A). The protein was eluted using an ascending linear gradient of 0–0.1 M NaCl in buffer A over a volume of 300 ml. The fractions collected were then loaded onto a HiLoad 26/60 Superdex 75 column (Amersham Biosciences) equilibrated with 0.2 M NaCl in buffer A. The purified protein was dialyzed overnight against 50 mM Tris buffer pH 8.8 and concentrated to 10 mg ml^{−1} for crystallization.

3. Crystallization

Crystallization condition screening was carried out using the Crystal Screen 1 and 2 kits from Hampton Research. Crystallization was performed at 289 K using the sitting-drop vapour-diffusion method on Greiner CrystalQuick plates. For each well, two drops were produced: one made up of 1 μ l each of reservoir solution and protein solution and the other made up of 1 μ l reservoir solution and 2 μ l protein solution. Crystals of the human common-type acylphosphatase grew in a broad range of conditions containing polyethylene glycol 4000 or 8000 as precipitants at pH 4.6–7.5. The best crystals were obtained by mixing 1 μ l 10% (v/v) 2-propanol, 20% (w/v) PEG 4000, 0.1 M Na HEPES buffer pH 7.5 (condition No. 41 of Crystal Screen 1) with 2 μ l 10 mg ml^{−1} protein sample. The crystals grew to maximum dimensions of $\sim 0.1 \times 0.1 \times 0.2$ mm in 2 d. Crystals that diffracted to a resolution of better than 1.5 Å were obtained directly from the screen and no further optimization of crystallization conditions was performed.

4. Preliminary X-ray diffraction data analysis

Crystals were cryoprotected by soaking the crystals in mother liquor with 20% (v/v) PEG 400 for 1 min. The crystal was then loop-mounted and placed in a stream of nitrogen at 100 K. X-ray diffraction data were collected on an R-Axis IV++ imaging-plate system using a rotating copper-anode X-ray source (Rigaku MicroMax-007 with VariMax optics). A total of 120 images were collected at 1° oscillations, with an exposure time of 10 min and a crystal-to-detector distance of 80 mm. The images were processed with *d*TREK* (Rigaku/MSO). Pseudo-precession images generated with *HKLVIEW* (Collaborative Computational Project, Number 4, 1994) revealed an *mmm* Laue symmetry. Reflection conditions $h = 2n, k = 2n, l = 2n$ were observed, indicating the presence of a twofold screw axis on all axes. The space group is $P2_12_12_1$, with unit-cell parameters $a = 42.58, b = 47.23, c = 57.26$ Å. Matthews coefficient (V_M) analysis (Matthews, 1968) suggests that the asymmetric unit contains one molecule of acylphosphatase, giving a V_M value of 2.6 Å³ Da^{−1} and a solvent content of 52%. Diffraction data were integrated and scaled to a maximum resolution of 1.45 Å; the statistics of data processing are summarized in Table 1.

Molecular replacement was performed with the program *MOLREP* (Collaborative Computational Project, Number 4, 1994) using all data in the resolution range up to 3.0 Å. All atoms from the crystal structure of bovine common-type acylphosphatase (PDB code 2acy; Thunnissen *et al.*, 1997) were included in the search model. The correlation coefficient and *R* factor of the solution were 0.517 and 0.455, compared with values of 0.144 and 0.609 for the next best solution. Model building and structure refinement are now under way.

This work was supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, China (project No. CUHK4590/05M) and a Direct Grant (project No. 2030324) from the Research Committee of the Chinese University of Hong Kong.

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