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Crystallization and preliminary crystallographic analysis of human common-type acylphosphatase

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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 acylphosphatases 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 *Drosophilia* (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)
$R_{\rm merge}$† (%)	3.2 (28.9)
Mean $I/\sigma(I)$	19.8 (2.9)

† $R_{\rm merge} = \sum_{hkl} |I - \langle I\rangle| / \sum_{hkl} I$.

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-thio-galactopyranose was added to a concentration of 0.2 m*M* to induce protein expression. After incubation at 310 K for 16–20 h, cells were harvested by centrifugation and resuspended in 20 m*M* Tris pH 7.5 with 0.5 m*M* phenylmethylsulfonyl fluoride and lysed by sonication. The lysed cells were centrifuged at 13 000*g* for 30 min. The supernatant was applied onto a HiTrap SP HP column (Amersham Biosciences) equilibrated with 20 m*M* Tris buffer pH 7.5 (buffer *A*). The protein was eluted using an ascending linear gradient of 0–0.1 m*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 m*M* 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 µl each of reservoir solution and protein solution and the other made up of 1 µl reservoir solution and 2 µl 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 µl 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 µl 10 mg ml^{-1} protein sample. The crystals grew to maximum dimensions of ~0.1 × 0.1 × 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/MSC). 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 ($V_{\rm M}$) analysis (Matthews, 1968) suggests that the asymmetric unit contains one molecule of acylphosphatase, giving a $V_{\rm M}$ value of 2.6 Å^3 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.

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