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Crystallization and preliminary X-ray crystallographic analysis of DFPase from *Loligo vulgaris*

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'Squid-type' diisopropylfluorophosphatases (DFPases), a subclass of the phosphotriesterases, are enzymes capable of hydrolysing organophosphorus nerve agents. To date, no three-dimensional structure of a 'squid-type' DFPase is known. Here, the crystallization of the DFPase originally isolated from head ganglion of the squid *Loligo vulgaris* is reported. The protein has been heterologously expressed in *Escherichia coli*, purified to homogeneity and subsequently crystallized. The protein crystals belong to space group $P2_12_12_1$, with unit-cell parameters a = 43.1, b = 82.1, c = 86.6 Å and one monomer per asymmetric unit. Under cryoconditions (120 K) the crystals diffracted beyond 2.0 Å using a Cu rotating-anode X-ray generator.

1. Introduction

Enzymes capable of hydrolyzing organophosphorus triesters, commonly termed phosphotriesterases (E.C. 3.1.8), have been found in a variety of organisms, *e.g.* in bacteria, protozoa, fungi, insects, fresh water clam, squid and mammals (see Dumas *et al.*, 1989), and more recently in birds (Monroy-Noyola *et al.*, 1999) and plants (Hoskin *et al.*, 1999). However, because of their large biochemical and sequence diversity, the organophosphorus-hydrolysing enzymes are not really comparable. One subclass of these phosphotriesterases, the diisopropylfluorophosphatases (DFPases; E.C. 3.1.8.2), are so named because of their ability to hydrolyse such nerve agents as soman, sarin, tabun and in particular diisopropylfluorophosphate (DFP). They are further subdivided into 'Mazur-type' DFPases (40–96 kDa) and 'squid-type' DFPases (35–40 kDa), which are distinguished by different soman and DFP hydrolysis rates (Hoskin *et al.*, 1984). Here, we report the crystallization and preliminary X-ray analysis of the 'squid-type' DFPase from *L. vulgaris*.

'Squid-type' DFPases have been detected in the head ganglia of several cephalopods, *e.g.* in *Octopus vulgaris*, *L. pealei*, *Todarodes pacificus steenstrup*, *L. opalescens* and *L. vulgaris* (Hoskin & Long, 1972; Wang *et al.*, 1993; Hoskin *et al.*, 1993; Deschamps *et al.*, 1993). The complete amino-acid sequence, however, is known only for the latter DFPase. Conformational similarities between the DFPases from *L. vulgaris* and *L. pealei* as well as between the DFPases from *L. pealei* and *L. opalescens* have been observed by studies with monoclonal antibodies, whereas no similarity with the 'Mazur-type' DFPase from *Pseudomonas diminuta* could be found (Hoskin *et al.*, 1993; Deschamps *et al.*, 1993). Obviously, the decontamination of organophosphorus nerve agents is not the physiological function of DFPases, since such compounds have only been designed synthetically in the last century. However, despite intensive studies, the actual role of DFPases from squid head ganglion still remains unclear.

The ability to hydrolyze nerve agents emphasises the importance of DFPases. Enzymatic hydrolysis provides a reasonable alternative to the currently discussed decontamination strategies of chemical warfare agents (Cheng *et al.*, 1999). Therefore, detailed information about the structure of these enzymes is crucial both for understanding the mechanistic aspects of organophosphate hydrolysis and for structure-based protein engineering aimed at altering hydrolysis rates and substrate specificity.

2. Materials and methods

2.1. Crystallization

The DFPase from *L. vulgaris* (314 residues, 35 kDa) has been heterologously expressed in *E. coli* using the expression plasmid pKKHisND and subsequently purified to homogeneity, as described in detail elsewhere (Hartleib & Rüterjans, 2000). Briefly, purification occurred *via* the following steps: metal-affinity chromatography on Ni^{2+}-NTA (Qiagen, Germany), rechromatography on Ni^{2+}-NTA after thrombin cleavage and a final anion-exchange chromatography on Q-Sepharose HP (Pharmacia, Sweden).

For crystallization experiments, 0.1–2 m*M* protein solutions in 10 m*M* Tris buffer pH 7.5,

2 m*M* $CaCl_2$ were used. Initial crystallization trials using the Crystal Screen I and II kits (Jancarik & Kim, 1991; Cudney *et al.*, 1994) and the Low Ionic Strength Screen kit (Harris *et al.*, 1995) (all from Hampton Research, CA, USA) revealed that the protein crystallizes under a variety of different conditions. At first, the orthorhombic crystals were not suitable for X-ray measurements owing to their small size, excessive twinning and low diffraction quality. Optimization of the crystallization parameters, however, improved the crystal quality considerably. The best results were finally obtained using the hanging-drop vapour-diffusion method in Linbro plates (Hampton Research, CA, USA) at 289 K with 12%(*w*/*v*) PEG 6000 and 0.1 *M* MES pH 6.5 as crystallization reagent. With 1 ml reservoir solution and droplets consisting of 2 µl protein solution (2 m*M*) and 2 µl crystallization reagent, crystals appeared within 2–3 d. The crystals grew to maximum dimensions of approximately 1.5 × 0.5 × 0.5 mm (Fig. 1).

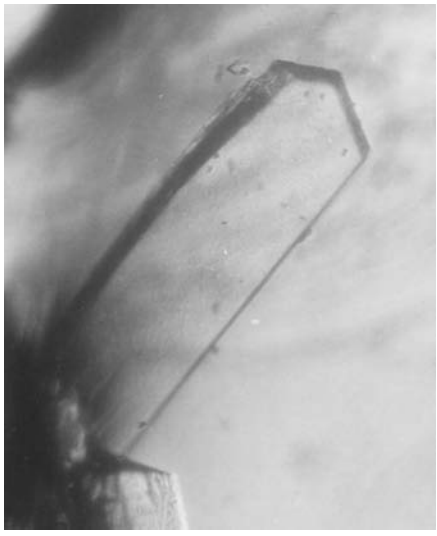

Figure 1
An orthorhombic crystal of DFPase grown in 12%(*w*/*v*) PEG 6000 and 0.1 *M* MES (pH 6.5). The dimensions of the crystal are approximately 0.7 × 0.2 × 0.1 mm.

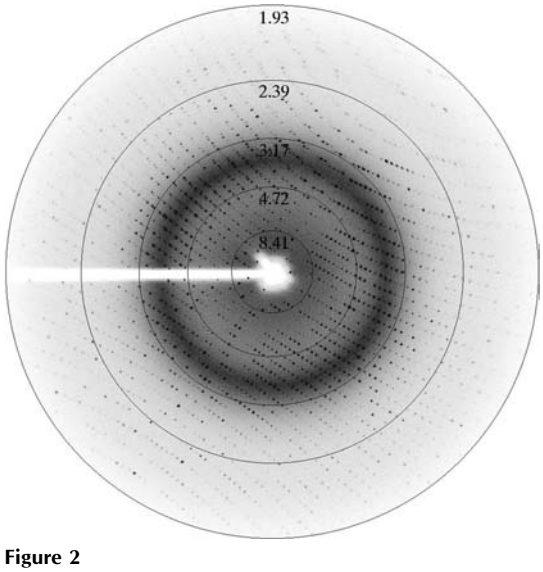

Figure 2
A 1.0° oscillation image of a DFPase crystal. The crystal diffracted beyond 2.0 Å resolution.

2.2. X-ray data collection and processing

Prior to X-ray data collection, the crystals were transferred to a cryoprotectant buffer consisting of reservoir solution supplemented with 20%(*v*/*v*) glycerol, picked up with a nylon loop and flash-cooled to 120 K in a stream of gaseous nitrogen from a cryocooling system (Oxford Cryosystems, England). X-ray diffraction data (Fig. 2) were collected using a Rigaku RU-200 rotating-anode X-ray generator equipped with a Cu anode (Cu $K\alpha$ radiation of 1.54180 Å wavelength, graphite monochromator, 0.3 mm collimator) and a 300 mm image-plate detector (MAR Research, Germany). The crystal-to-detector distance was set to 140 mm, equivalent to 1.93 Å maximum resolution at the edge of the image plate. 153 rotation images were collected with a rotation angle of 1.0° and an exposure time of 1500 s. Using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997) for data reduction and scaling, the raw data were processed to a resolution of 2.0 Å.

3. Results

Based on the data set summarized in Table 1, the space group was determined to be $P2_12_12_1$, with unit-cell parameters a = 43.1, b = 82.1, c = 86.6 Å. From the crystal volume per protein mass (V_M) of 2.18 $Å^3\ Da^{-1}$, it can be concluded that the asymmetric unit contains one DFPase molecule. The larger crystals displayed high mosaicity (up to 1.0), but smaller crystals of about 0.5 × 0.2 × 0.1 mm in size were in an acceptable mosaicity range (0.4–0.5).

The next step will be the measurement of a high-resolution data set with a smaller crystal at a synchrotron X-ray source. Since there are no protein structures available that show significant sequence homology with the DFPase from *L. vulgaris*, recombinant expression in *E. coli* will be utilized to obtain a selenomethionine-modified protein. The structure will subsequently be determined using the multiple-wavelength anomalous dispersion (MAD) approach.

Table 1
X-ray data-collection statistics.

Values in parentheses refer to the highest resolution shell.

No. of observations	100131
No. of unique reflections	20720 (866)
Multiplicity	4.8
Resolution range (Å)	30–2.00 (2.03–2.00)
Completeness (%)	95.6 (80.9)
Average $I/\sigma(I)$	8.0 (2.1)
R_{sym}† (%)	7.3 (17.0)
Mosaicity (°)	0.9

† $R_{sym} = \sum_{hkl}\sum_i |I_i - \langle I\rangle| / \sum \langle I\rangle$, where I_i is the intensity of the ith measurement of reflection hkl and $\langle I\rangle$ is the average intensity of a reflection.

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