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Crystallization and X-ray diffraction of 5′-fluoro-5′-deoxyadenosine synthase, a fluorination enzyme from *Streptomyces cattleya*

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Organofluorine compounds are widely prepared throughout the chemicals industry, but their prepararion generally requires harsh fluorinating reagents and non-aqueous solvents. On the other hand, biology has hardly exploited organofluorine compounds. A very few organisms synthesize organofluorine metabolites, suggesting they have evolved a mechanism to overcome the kinetic desolvation barrier to utilizing F^-(aq). Here, the purification and crystallization of an enzyme from *Streptomyces cattleya* which is responsible for the synthesis of the C—F bond during fluoroacetate and 4-fluorothreonine biosynthesis is reported. The protein crystallizes in space group $C222_1$, with unit-cell parameters a = 75.9, b = 130.3, c = 183.4 Å, $\alpha = \beta = \gamma = 90^\circ$. Data were recorded to 1.9 Å at the ESRF. The structure of the protein should provide important insights into the biochemical process of C—F bond formation.

1. Introduction

Organofluorine compounds are widely used in both medicine and agriculture. The substitution of F for H is a common variation in structure–activity relationships of candidate drugs. Although a straightforward strategy, the actual synthesis of such substituted molecules is often difficult and typically employs highly reactive and noxious reagents such as F_2, SF_4, HF and CoF_3. In part, this is because fluoride in the gas phase is reactive, but in water it is so tightly solvated ($\Delta G^\circ > 450$ kJ mol^{-1}) as to be kinetically inert. Thus, an enzymatic transformation to make organofluorine compounds is highly desired. A small group of organofluorine compounds have been identified from plants and microorganisms (O'Hagan & Harper, 1999), suggesting biology has indeed found a solution to overcome the inert-like behaviour of F^-(aq). Of particular note is the identification of fluoroacetic acid in the South African plant *Dichapetalum cymosum* (O'Hagan *et al.*, 1993) and in the microorganism *Streptomyces cattleya* (Sanada *et al.*, 1986). Although over 50 years have passed since the first organofluorine metabolite was identified in *D. cymosum*, the mechanism for enzymatic formation of the C—F bond has remained elusive. Analogy with the haloperoxidases which generate Cl^+ or Br^+ species by oxidation of the anion with hydrogen peroxide (Littlechild, 1999; van Pee, 1996) cannot be extended to fluorine as peroxide is incapable of oxidizing F^- to F^+. The mechanism by which fluorine is introduced into organic compounds in biology remains unknown, but clearly an enzymatic process will reveal exciting biotechnological possibilities. It may also illuminate new mechanistic strategies for organofluorine synthesis.

The first native fluorination enzyme (5′-fluoro-5′-deoxyadenosine synthase) was recently identified from *S. cattleya* and has been shown to catalyse formation of a C—F bond (O'Hagan *et al.*, 2002). The 32 kDa enzyme utilizes *S*-adenosylmethionine (SAM) and F^-(aq) as substrates. The products are methionine and 5′-fluoro-5′-deoxyadenosine (O'Hagan *et al.*, 2002) (Fig. 1). A stereo-

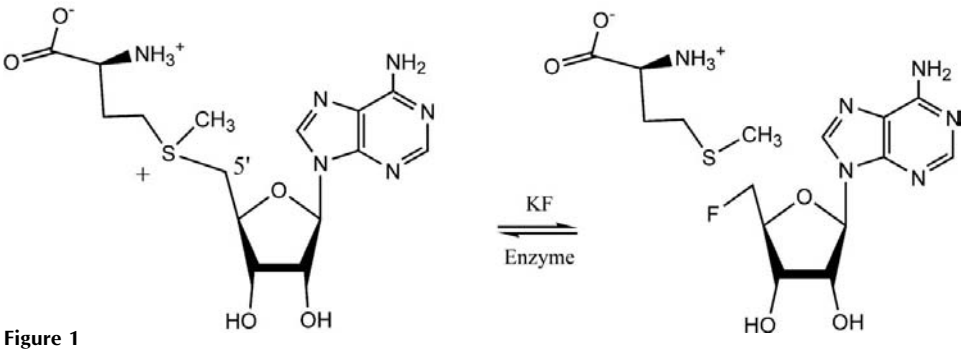

Figure 1
The reaction between fluoride ion and SAM catalysed by 5′-fluoro-5′-deoxyadenosine synthase.

chemical study suggests that the enzyme catalyses the S_N2 displacement of L-methionine by nucleophilic attack at C5′ of adenosine (O'Hagan *et al.*, 2003). Clearly, the enzyme must desolvate the F^- ion to some degree at the catalytic site in order to overcome the reactivity problem. Here, we report the purification and crystallization of 5′-fluoro-5′-deoxyadenosine synthase from *S. cattleya*. Data were collected from a single frozen crystal to 1.9 Å. This work will form the basis of the structure determination of this natural fluorinating enzyme, which will allow us to elucidate for the first time details of biological fluorination.

2. Materials and methods

2.1. Isolation and purification

200 ml cultures of *S. cattleya* (strain NRRL 8057) were grown in 2 l shake flasks at 301 K with KF added to a final concentration of 2 m*M* according to a published protocol (Schaffrath *et al.*, 2003) for 7 d. The cells were harvested by centrifugation and lysed by sonication in 100 m*M* Tris pH 7.8 containing the protease inhibitors leupeptin, pepstain, aprotinin and phenylmethylsulfonyl fluoride (Roche). Cell debris was removed by centrifugation and ammonium sulfate was added to the supernatant to 45% saturation. The precipitate was removed by centrifugation and additional ammonium sulfate was added to a final concentration of 60% saturation and the precipitant retained. A buffer of 50 m*M* Tris pH 7.8 was used in all purification stages. The pellet was resuspended in buffer containing 1.2 *M* ammonium sulfate and centrifuged. The supernatant was loaded onto a HP hydrophobic affinity column (Perseptive Biosystems) and eluted using a decreasing gradient of ammonium sulfate (1.2–0 *M*) over 12 column volumes. The protein was further purified on a Superdex-200 column (Amersham Biosciences) with a final step on a HQ anion-exchange column (Perseptive Biosystems) with a KCl gradient (0–1 *M*) over 12 column volumes. Analysis by gel filtration suggested that the protein ran consistent with a hexameric organization (186 kDa). The protein was dialysed against 25 m*M* Tris–HCl pH 7.8 and concentrated to 5 mg ml^{-1} for crystallization. A yield of 1 mg per litre of culture was obtained. Visualization of the protein by sodium dodecylsulfate electrophoresis showed a single band, even when 5′-fluoro-5′-deoxyadenosine synthase was overloaded. Protein integrity was confirmed by mass spectroscopy and by enzyme assay (Schaffrath *et al.*, 2002).

2.2. Crystallization and data collection

The protein was tested for crystallization against a collection of commercial screens and grids (Hampton Research, Wizard and Index) by sitting-drop vapour diffusion at 293 K. Each well contained 2 µl of protein and 2 µl of precipitant and a reservoir containing 100 µl of precipitant. Crystals appeared in a number of conditions. The highest quality crystals of the enzyme were obtained by vapour diffusion against 22% PEG 1000, 0.1 *M* phosphate–citrate pH 4.2, 0.2 *M* Li_2SO_4. The crystals are almost tetragonal, with dimensions of 0.5 × 0.3 × 0.3 mm, and are shown in Fig. 2. The crystal could be flash-frozen in a cryoprotectant consisting of 40% PEG 1000, 0.1 *M* phosphate–citrate pH 4.2, 0.2 *M* Li_2SO_4 at 100 K. Data to 1.9 Å were collected at ESRF beamline ID14-2 using two passes. The first high-resolution pass (6–1.9 Å) collected 210 frames of 1° oscillation with a crystal-to-detector distance of 160 mm and 15 s exposure per frame. A second lower resolution pass (61–2.6 Å) of 210 1° oscillations was collected with a crystal-to-detector distance of 250 mm and 3 s exposure. The data were indexed and integrated using *MOSFLM* (Collaborative Computational Project, Number 4, 1994; Leslie, 1992) and the two data sets were scaled together using *SCALA* (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

Indexing of the crystals identified them as *C*-centred orthorhombic. Examination of systematic absences suggested that the space group was $C222_1$. The unit-cell parameters were $a = 75.9$, $b = 130.3$, $c = 183.4$ Å, $\alpha = \beta = \gamma = 90°$. Interestingly, despite the tetragonal appearance of the crystals the 130 Å cell edge aligns along the long axis of the crystal. Full details of the X-ray data set are listed in Table 1. The asymmetric unit volume is 226 000 Å^3 and is consistent with a trimer in the asymmetric unit (Matthews coefficient of 2.5 Å^3 Da^{-1}; 45% solvent). If the protein is a hexamer then one of the crystallographic twofold axes must generate the hexamer. Inspection of the self-rotation function using *CCP*4 (Collaborative Computational Project, Number 4, 1994) reveals a peak (approximately 20% of the origin) at $\omega = 8$, $\varphi = 0$, $\kappa = 120°$ consistent with a threefold non-crystallographic axis. In the absence of a gene sequence, structure solution will rely on conventional heavy-atom derivatization. However, work aimed at determining the gene sequence is ongoing and we expect to be able to employ selenomethionine multiwavelength methods.

Figure 2
Crystal of 5′-fluoro-5′-deoxyadenosine synthase.

Table 1
Summary of data-collection statistics.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	0.932
Resolution (Å)	65–1.9 (1.98–1.90)
Space group	$C222_1$
Unit-cell parameters (Å, °)	$a = 75.9$, $b = 130.3$, $c = 183.4$, $\alpha = \beta = \gamma = 90$
No. unique reflections	67204
Multiplicity	10.0 (7.7)
$I/\sigma(I)$	4.8 (1.7)
Completeness (%)	94 (93)
$R_{\rm merge}$† (%)	11.0 (44.0)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i - \langle I\rangle| / \sum_{hkl}\sum_i \langle I\rangle$, where I_i is the intensity of the ith measurement of a reflection with indices hkl and $\langle I\rangle$ is the weighted mean of the reflection intensity.

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