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# Preliminary crystallographic studies of the creatinine amidohydrolase from *Pseudomonas putida*

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Creatinine amidohydrolase (creatininase; EC 3.5.2.10) from *Pseudomonas putida* has been overexpressed in *Escherichia coli* and crystallized by the hanging-drop method. The crystal belongs to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a$ = 102.0, $b$ = 150.7, $c$ = 167.1 Å. Native data were collected to 1.8 Å resolution by a rotation method at 100 K using an ADSC Quantum 4R CCD detector with synchrotron radiation.

## 1. Introduction

Creatinine amidohydrolase (creatininase; EC 3.5.2.10) catalyzes the reversible conversion of creatinine to creatine. The enzyme was found in *Pseudomonas putida* (Tsuru *et al.*, 1976) and purified to homogeneity (Rikitake *et al.*, 1979). It is used as a key enzyme for the enzymatic measurement of creatinine by coupling with creatine amidinohydrolase (EC 3.5.3.3; Yoshimoto *et al.*, 1976) and sarcosine dehydrogenase (EC 1.5.3.1; Oka *et al.*, 1979). Diagnostic analysis of creatinine in serum and urine is important for assessing renal function. The nucleotide sequence of creatininase from *P. putida* has been determined (Yamamoto *et al.*, 1995). The amino-acid sequence deduced from the nucleotide sequence showed 65 and 37% identity to creatininases from *Alcaligenes* sp. (Nishiya *et al.*, 1998) and *Arthrobacter* sp. (Nishiya *et al.*, 2001), respectively. There are 16 enzyme groups that are classified as hydrolases acting on carbon–nitrogen bonds in cyclic amides (EC 3.5.2.*x*). Three-dimensional structures have been clarified for only two enzymes, dihydroorotase (PDB code 1j79) and $\beta$-lactamase (PDB code 1a7t), of the enzymes listed in EC 3.5.2.*x*. Since there is no study of creatininase, we performed the crystallization and preliminary X-ray crystallographic analysis of the enzyme from *P. putida*.

## 2. Crystallization

The creatininase gene (780 bp) from *P. putida* was inserted into pBluscript SK(+) and overexpressed in *Escherichia coli*. The enzyme was purified to homogeneity (Yamamoto *et al.*, 1995). The purity of the sample was more than 99% as judged by SDS–PAGE. A hanging drop was prepared by mixing 5 µl each of the enzyme solution (protein concentration 35 mg ml$^{-1}$) and 1.5 *M* lithium sulfate in 0.1 *M* Na HEPES pH 7.5. Tetragonal crystals appeared in 2 d at 297 K and reached dimensions of 0.5 × 0.5 × 0.6 mm after one week (Fig. 1); these crystals were used to collect data.

## 3. X-ray data collection

As creatininase crystals showed significant radiation damage during data collection at 293 K, data collection was performed under cryogenic conditions. For data collection under cryogenic conditions, crystals in a droplet were transferred through a series of harvesting solutions (1.5 *M* lithium sulfate in 0.1 *M* Na HEPES buffer pH 7.5) in which the concentration of glycerol was increased successively in three steps, starting at 0%(*v*/*v*) for more than 3 min, increasing to 10%(*v*/*v*) for 1 min and to a final concentration of 20%(*v*/*v*) for 1 min. Crystals were mounted on nylon loops and flash-frozen in a cold nitrogen-gas stream at 100 K just prior to data collection. Data collection was performed by a rotation method

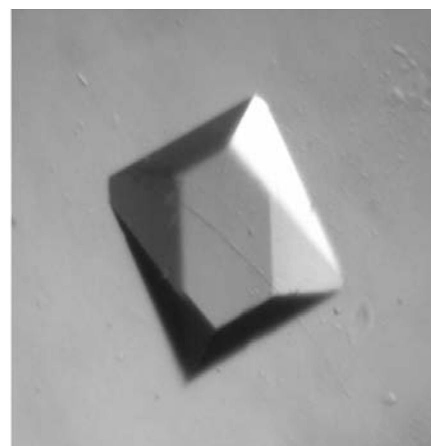

**Figure 1**
A crystal of creatinine amidohydrolase as grown by the hanging-drop method. The average dimensions of the crystals were 0.5 × 0.5 × 0.6 mm.

at 100 K using an ADSC Quantum 4R CCD detector with synchrotron radiation ($\lambda$ = 0.978 Å at beamline 6A of the Photon Factory, Tsukuba, Japan). The Laue group and unit-cell parameters were determined using the *DPS* program package (Rossmann & van Beek, 1999). The Laue group was found to be *mmm* and the unit-cell parameters were $a$ = 102.0 (1), $b$ = 150.7 (1), $c$ = 167.1 (1) Å. Only reflections with $h = 2n$, $k = 2n$ and $l = 2n$ were observed along the ($h$00), (0$k$0) and (00$l$) axes, respectively, indicating the orthorhombic space group $P2_12_12_1$. Assuming six or 12 subunits (one or two hexamers) per asymmetric unit leads to empirically acceptable $V_M$ values of 3.8 or 1.9 Å$^3$ Da$^{-1}$ and corresponding solvent contents of 68 or 35%, respectively (Matthews, 1968). The current best diffraction data from a native crystal were collected to 1.8 Å resolution and were processed with the program packages *DPS* (Rossmann and van Beek, 1999) and *CCP*4 (Collaborative Computational Project, Number 4, 1994) (Table 1). Although the creatininase crystal diffracted to at least 1.8 Å, its relatively large unit-cell size and the limited size of the detector area made high-resolution data collection difficult.

**Table 1**
Crystallographic data for creatininase.

Values in square brackets are for the highest resolution shell.

| | |
|---|---|
| Wavelength (Å) | 0.978 |
| Space group | $P2_12_12_1$ |
| Unit-cell parameters (Å) | $a$ = 102.0 (1), $b$ = 150.7(1), $c$ = 167.1 (1) |
| Resolution (Å) | 32–1.8 [1.90–1.80] |
| Observed reflections | 1294342 |
| Unique reflections | 237380 |
| Mean $\langle I/\sigma(I)\rangle$ | 7.4 [2.1] |
| $R_{merge}$ (%) | 6.3 [33.1] |
| Completeness (%) | 99.9 [99.5] |

Preliminary phases for creatininase crystals have been obtained by a multiple-wavelength anomalous dispersion experiment around the $K$ absorption edge of intrinsic zinc ions (at beamline 18B of the Photon Factory). There are six zinc sites in an asymmetric unit and the six sites are related by 32 ($D_3$) point-group symmetry. Creatininase contains one zinc ion per subunit (Rikitake *et al.*, 1979), indicating that there are six subunits (one hexamer) in the asymmetric unit. A full description of the structure determination of creatininase will be published elsewhere.

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