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# Crystallization and preliminary X-ray crystallographic analysis of a putative agmatinase from *Deinococcus radiodurans*

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Agmatine, which results from the decarboxylation of arginine by arginine decarboxylase, is a metabolic intermediate in the biosynthesis of putresine and higher polyamines. The enzyme agmatinase catalyses the hydrolysis of agmatine to putresine and urea. Recent studies indicate that agmatinase plays important roles in mammals. Human mitochondrial agmatinase shows a considerable level of sequence similarity to bacterial agmatinases, including a putative agmatinase from *Deinococcus radiodurans*. The putative agmatinase from *D. radiodurans* has been overexpressed in *Escherichia coli* and crystallized at 297 K using polyethylene glycol 3000 as a precipitant. X-ray diffraction data were collected to 1.80 Å from a crystal grown in the presence of $Mn^{2+}$ and 1,6-hexanediamine. The crystals are orthorhombic, belonging to the space group $P2_12_12_1$, with unit-cell parameters $a$ = 81.77, $b$ = 131.44, $c$ = 168.85 Å, $\alpha = \beta = \gamma = 90^\circ$. A hexameric molecule is likely to be present in the asymmetric unit, giving a crystal volume per protein weight ($V_M$) of 2.15 Å$^3$ Da$^{-1}$ and a solvent content of 41.8%.

## 1. Introduction

Agmatine is an amine that is synthesized by decarboxylation of L-arginine by arginine decarboxylase (ADC; Reis & Regunathan, 2000). In bacteria, plants and invertebrates, agmatine is hydrolyzed to putrescine, the precursor of spermine, by agmatinase (agmatine ureohydrolase, EC 3.5.3.11; Reis & Regunathan, 2000). In 1994, agmatine was identified as an endogenous ligand for the imidazoline receptor and agmatinase and ADC were subsequently identified in mammalian brain. Several lines of evidence indicate that agmatinase may play important roles in mammals, including the regulation of neurotransmitter-related functions (Reis & Regunathan, 1998, 2000). The amino-acid sequences of agmatinases indicate their homology to arginases, suggesting a common evolutionary origin (Ouzounis & Kyrpides, 1994; Perozich *et al.*, 1998). Compared with the wealth of structural data on arginases, little structural information is available on agmatinases. The human agmatinase gene encodes a 352-residue protein with a putative mitochondrial targeting sequence at the amino-terminus. Human agmatinase has about 30% sequence identity to bacterial agmatinases and <20% identity to mammalian arginases. Residues required for binding of $Mn^{2+}$ at the active site in bacterial agmatinases and other members of the arginase superfamily are fully conserved in human agmatinase (Mistry *et al.*, 2002; Iyer *et al.*, 2002).

In order to understand the functional differences between agmatinase and arginase in structural terms and to better understand the functional roles of agmatinase, we have initiated three-dimensional structure determination of agmatinase. In this study, we have overexpressed a putative agmatinase from *Deinococcus radiodurans*, a 304-residue protein of 35 751 Da, in *Escherichia coli* and have crystallized it. Of the protein sequences in the SWISS-PROT database, this enzyme shows the highest level of sequence identity to human mitochondrial agmatinase; the sequence identity is 37% over the region encompassing residues 11–298. We report here its crystallization conditions and preliminary X-ray crystallographic data.

## 2. Experimental

### 2.1. Protein expression and purification

The putative agmatinase gene (DR0149) was amplified by the polymerase chain reaction using the *D. radiodurans* genomic DNA as template. The forward and reverse oligonucleotide primers designed using the published genome sequence (White *et al.*, 1999) were 5′-G GAA TTC **CAT ATG** AGC GGG CCG GCC CAC C-3′ and 5′-CCG CCG **CTC GAG** GAC ATG GTC GAA CAC CTC GCA C-3′, respectively, where the bases in bold represent the *Nde*I and *Xho*I restriction-enzyme cleavage sites. The amplified DNA was inserted into the *Nde*I/*Xho*I-digested expres-

sion vector pET-28b(+) (Novagen). This vector construction adds an eight-residue tag (LEHHHHHH) to the carboxyl-terminus and a 20-residue tag (MGSSHHHHHHSSGLVPRGSH) to the amino-terminus of the gene product in order to facilitate protein purification. The protein was overexpressed in *E. coli* C41(DE3) cells (Miroux & Walker, 1996). Cells were grown at 310 K to an $OD_{600}$ of 0.5 in Terrific Broth medium containing 50 µg ml$^{-1}$ kanamycin and protein expression was induced by 1.0 m*M* isopropyl-$\beta$-D-thiogalactopyranoside (IPTG). Cell growth continued at 293 K for 18 h after IPTG induction and cells were harvested by centrifugation at 4200*g* (6000 rev min$^{-1}$; Sorvall GSA rotor) for 10 min at 277 K. The cell pellet was resuspended in ice-cold lysis buffer (20 m*M* Tris–HCl pH 7.9, 500 m*M* sodium chloride, 5 m*M* imidazole and 1 m*M* phenylmethylsulfonyl fluoride) and homogenized with an ultrasonic processor. The crude lysate was centrifuged at 70 400*g* (30 000 rev min$^{-1}$; Beckman 45Ti rotor) for 1 h at 277 K and the recombinant protein in the supernatant fraction was purified in two chromatographic steps. The first step utilized the hexahistidine tags by metal-chelate chromatography on Ni–NTA resin (Qiagen). Next, gel filtration was performed on a HiLoad XK 16 Superdex 200 prep-grade column (Amersham Pharmacia) previously equilibrated with buffer *A* (50 m*M* Tris–HCl pH 7.5) containing 100 m*M* sodium chloride and 1 m*M* manganese sulfate. The homogeneity of the purified protein was assessed by SDS–PAGE (Laemmli, 1970). The protein solution was concentrated using an YM10 ultrafiltration membrane (Amicon). The protein concentration was estimated by measuring the absorbance at 280 nm, employing the calculated extinction coefficient of 16 500 $M^{-1}$ cm$^{-1}$ (SWISS-PROT; http://www.expasy.ch/).

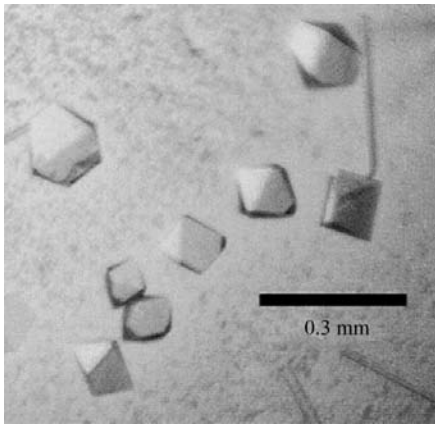


**Figure 1**
Crystals of the putative agmatinase from *D. radiodurans*. Their approximate dimensions are 0.15 × 0.15 × 0.20 mm.

**Table 1**
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (1.86–1.80 Å).

| | |
|---|---|
| X-ray wavelength (Å) | 1.000 |
| Temperature (K) | 100 |
| Space group | $P2_12_12_1$ |
| Unit-cell parameters (Å) | $a$ = 81.77, $b$ = 131.44, $c$ = 168.85 |
| Resolution range (Å) | 30.0–1.80 |
| Total/unique reflections | 713493/164504 |
| $R_{\rm merge}$† (%) | 7.1 (39.6) |
| Data completeness (%) | 98.0 (94.5) |
| Average $I/\sigma(I)$ | 22.4 (3.3) |

† $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \langle I(h)\rangle$, where $I(h)i$ is the intensity of the $i$th measurement of reflection $h$ and $\langle I(h)\rangle$ is the mean value of $I(h)$ for all $i$ measurements.

### 2.2. Crystallization and dynamic light scattering

Crystallization was performed at 297 K by the hanging-drop vapour-diffusion method using 24-well tissue-culture plates. Each hanging drop was prepared on a siliconized cover slip by mixing equal volumes (6 µl each) of the protein solution at 1.4 m*M* concentration and the inhibitor 1,6-hexanediamine solution at 334 m*M* concentration, resulting in an approximately 1:6.7 molar ratio of the agmatinase monomer to inhibitor. Each hanging drop was placed over 1.0 ml reservoir solution. Initial crystallization conditions were established using screening kits from Hampton Research (Crystal Screens I and II and MembFac) and from deCODE Biostructures Group (Wizard I and II). Dynamic light-scattering experiments were performed using a model DynaPro-801 instrument from Protein Solutions (Lakewood, New Jersey). The data were measured at 297 K with the protein at 1 mg ml$^{-1}$ concentration in 50 m*M* Tris–HCl pH 7.5, 100 m*M* sodium chloride and 1 m*M* manganese sulfate in the presence of a 6.7-fold molar excess of 1,6-hexanediamine.

### 2.3. X-ray diffraction experiment

Crystals were flash-frozen using a cryoprotectant solution consisting of 100 m*M* sodium phosphate citrate pH 4.2, 200 m*M* sodium chloride, 12%(*w*/*v*) PEG 3000 and 15%(*v*/*v*) glycerol. Crystals were soaked in 5 µl of the cryoprotectant solution for 10 s before being flash-frozen in liquid nitrogen. X-ray diffraction data were collected at 100 K using a DIP-2030 image-plate detector (MacScience) at beamline BL-6B of Pohang Light Source, South Korea. The crystal was rotated through a total of 180° with a 1.0° oscillation range per frame. The wavelength of the synchrotron radiation was 1.000 Å. The raw data were processed and scaled using the *HKL* program package (Otwinowski & Minor, 1997).

## 3. Results

When the recombinant agmatinase from *D. radiodurans* was expressed in *E. coli* as a fusion with the C-terminal eight-residue tag (LEHHHHHH), the expression level was very low. However, when the 20-residue tag (MGSSHHHHHHSSGLVPRGSH) was also fused at the N-terminus of the protein, it was highly overexpressed in a soluble form, with a much higher yield of ∼40 mg of purified enzyme per litre of culture. Dynamic light-scattering analysis indicated the purified protein to be monodisperse with a polydispersity of 20.5% and a native molecular weight of ∼185 000 Da. This result is consistent with *D. radiodurans* agmatinase existing as a tetramer, pentamer or hexamer in solution (the calculated monomer weight including the N- and C-terminal tags is 35 751 Da).

Despite the presence of both N- and C-terminal tags, the recombinant enzyme readily formed well diffracting crystals. The best crystals were obtained with a reservoir solution comprising 100 m*M* sodium phosphate–citrate pH 4.2, 200 m*M* sodium chloride, 12%(*w*/*v*) PEG 3000 and 30 m*M* glycyl-glycyl-glycine. Rectangular crystals grew to maximum dimensions of 0.15 × 0.15 × 0.20 mm within two weeks (Fig. 1). A flash-frozen crystal diffracted to ∼1.8 Å. A set of diffraction data was collected with a completeness of 98.0% to 1.80 Å and an $R_{\rm merge}$ of 7.1%. Table 1 summarizes the statistics of data collection. The space group was determined to be $P2_12_12_1$ on the basis of systematic absences and the unit-cell parameters are $a$ = 81.77 (4), $b$ = 131.44 (3), $c$ = 168.85 (3) Å, $\alpha = \beta = \gamma = 90°$, where estimated standard deviations are given in parentheses. If it is assumed that a hexameric molecule of the recombinant enzyme is present in the crystallographic asymmetric unit, the crystal volume per protein weight ($V_{\rm M}$) is 2.15 Å$^3$ Da$^{-1}$ and the solvent content is 41.8% by volume (Matthews, 1968). This assumption is supported by the self-rotation function, which clearly indicated the presence of threefold rotational symmetry. Hence, the hexameric molecule must have 32 symmetry. The structure has been determined by

molecular replacement and the details will be described elsewhere.

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