Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary crystallographic analysis of a D-aminoacylase from *Alcaligenes faecalis* DA1

Cheng-Sheng Hsu,[a,b] Shen-Jia Chen,[a] Ying-Chieh Tsai,[a] Ting-Wan Lin,[c] Shwu-Huey Liaw[a,d,e]*† and Andrew H.-J. Wang[c]†

[a]Institute of Biochemistry, National Yang-Ming University, Taipei, Taiwan, [b]Department of Biochemistry, National Taipei College of Nursing, Taipei, Taiwan, [c]Institute of Biological Chemistry, Academia Sinica, Taiwan, [d]Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan, and [e]Department of Life Science, National Yang-Ming University, Taipei, Taiwan

† These authors contributed equally in the supervision of this paper.

Correspondence e-mail: shliaw@ym.edu.tw

Received 22 April 2002
Accepted 19 June 2002

D-Aminoacylases catalyze the hydrolysis of *N*-acyl-D-amino acids into D-amino acids with the aid of zinc ions. The first D-aminoacylase crystal from *Alcaligenes faecalis* has been obtained in hanging drops at pH 5.6 by the vapour-diffusion method using 30% polyethylene glycol 4000 as precipitant. It belongs to space group $P2_12_12_1$, with unit-cell parameters a = 60.2, b = 76.6, c = 135.3 Å. Reflections to 1.2 Å resolution are observable. An initial atomic model with 472 residues has been built based on SeMet SAD data at 1.8 Å resolution. Unexpectedly, the structure revealed a novel metal centre in the amidohydrolase superfamily.

1. Introduction

N-acyl-D-amino acid amidohydrolases (D-aminoacylases) catalyze the hydrolysis of *N*-acyl-D-amino acids to produce the corresponding D-amino acids, which are intermediates in the preparation of pesticides, bioactive peptides and antibiotics. Several D-aminoacylases screened from microorganisms in various soils have been isolated and characterized (Sugie & Suzuki, 1978; Kubo *et al.*, 1980; Yang *et al.*, 1991; Tsai *et al.*, 1992; Moriguchi *et al.*, 1993). The D-aminoacylase from *Alcaligenes faecalis* DA1 shares high sequence identity to the D-aminoacylase (85%), *N*-acyl-D-aspartate amidohydrolase (56%) and *N*-acyl-D-glutamate amidohydrolase (47%) from *A. xylosoxydans* A-6 and the D-aminoacylase (39%) from *Pyrococcus abyssi* (Wakayama, Ashika *et al.*, 1995; Wakayama, Katsuno *et al.*, 1995; Wakayama, Watanabe *et al.*, 1995; Hsu *et al.*, 2002). However, it does not show any significant homology with L-aminoacylase.

The D-aminoacylases are zinc enzymes and removal of the bound zinc ions completely abolishes the catalytic activity (Yang *et al.*, 1992; Wakayama, Katsuno *et al.*, 1995). Recently, the putative metal-chelating ligands were identified based on structural prediction (Hsu *et al.*, 2002) and mutational studies (Wakayama *et al.*, 2000; Hsu *et al.*, 2002). D-Aminoacylases may belong to the recently identified amidohydrolase superfamily, in which only the metal ligands, four histidines and one aspartate, are strictly conserved (Holm & Sander, 1997). To understand the structural details of the metal-assisted catalysis, we have obtained the first D-aminoacylase crystal from *A. faecalis* DA1 and solved the structure using a SeMet SAD data set at 1.8 Å resolution.

2. Protein preparation, crystallization and X-ray data analysis

The 483-residue *A. faecalis* DA1 D-aminoacylase was expressed in *Escherichia coli* M15 and isolated by four chromatographic procedures as described elsewhere (Hsu *et al.*, 2002). The recombinant protein contains 13 additional vector residues at the N-terminus. To express the SeMet protein, the recombinant expression vector pQE30 (Qiagen) was transformed into *E. coli* B834 (DE3). After incubation overnight at 310 K in LB medium containing 100 µg ml^{-1} ampicillin, the bacteria were then used to inoculate 7 l of LeMaster Medium supplemented with 50 mg l^{-1} seleno-DL-methionine (Hendrickson *et al.*, 1990). Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.75 m*M* for induction after the cell density reached an A_{600} of 0.5. The cells were grown for another 10 h at 310 K and then harvested by centrifugation. The SeMet protein was purified as for the native protein.

The initial crystallization screening was performed with Hampton Research Crystal Screens using the hanging-drop vapour-diffusion method at 295 and 277 K. Crystals suitable for X-ray diffraction analysis were grown in 25–30% polyethylene glycol 4000, 0.1 *M* sodium citrate pH 5.6 and 0.2 *M* ammonium acetate, with a combination of 2 µl of protein solution (12–20 mg ml^{-1}) and 2 µl of reservoir solution. Crystals appeared and reached their final sizes in a week at 295 K and in 2–4 weeks at 277 K (Fig. 1). The SeMet protein was crystallized under the same conditions.

The crystal was scooped up in a cryoloop and directly frozen in liquid nitrogen without addition of any other cryoprotectants. It was then mounted on the goniometer in a nitrogen

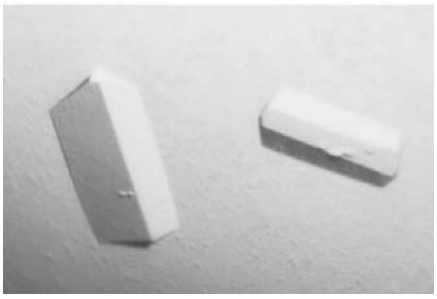

Figure 1
Typical crystals of D-aminoacylase, with dimensions of 0.4 × 0.2 × 0.15 mm.

stream at 100 K. Preliminary X-ray diffraction experiments were carried out with an R-AXIS IV image-plate detector (MSC) using Cu $K\alpha$ radiation generated by a Rigaku RU-300 rotating-anode generator. Data at higher resolution were collected using an ADSC Quantum 4 CCD camera at beamlines 6A and 18B of the Photon Factory of the High Energy Accelerator Research Organization (Watanabe *et al.*, 1995). Data were processed with the program *MOSFLM* (Leslie, 1997).

The phase problem was solved by using the program *CNS* (Brünger *et al.*, 1998). After a heavy-atom search and density modification, the electron-density map was submitted to the automatic *wARP* procedure (Perrakis *et al.*, 1999). The initial atomic model was subsequently refined using *CNS* and *O* (Jones & Kjeldgaard, 1997).

3. Results and discussion

Crystals could be obtained from the recombinant protein purified using either four columns or an Ni–NTA (Qiagen) column only. However, the purification profile of the SeMet protein was quite different from the native protein. Both Ni–NTA and HiTrap Q (Pharmacia) columns were required for higher protein purity suitable for crystallization.

Even in the same crystallization drops, some crystals were too mosaic to collect data from. Crystals suitable for data collection were therefore screened. Autoindexing and consideration of systematically absent reflections revealed that the crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 60.2, b = 76.6, c = 135.3 Å. The packing density suggests that there is one molecule in an asymmetric unit (V_M = 2.9 Å^3 Da^{-1}), with a solvent content of 57%. The crystals diffract beyond 1.2 Å resolution using synchrotron radiation. A single SeMet SAD data set has been collected at the peak wavelength of 0.9787 Å. The data-collection statistics are summarized in Table 1.

Table 1
Summary of data-collection statistics.

Values in parentheses are for the highest resolution shell.

Resolution (Å)	1.8 (1.9–1.8)
Total observations	360069
Unique reflections	56888
Completeness (%)	98.3 (94.4)
Anomalous completeness (%)	92.4 (80.7)
$\langle I/\sigma(I)\rangle$	12.2
Multiplicity	6.3 (5.8)
R_{merge} (%)	5.1 (15.7)
R_{anom} (%)	6.2 (11.0)

A virtually complete atomic model with 472 residues was obtained in an almost automatic manner by the sequential use of the programs *CNS* and *wARP*. The recombinant protein contains 14 methionines. 12 Se peaks were identified and the first two methionines in the vector were disordered (Fig. 2). Unexpectedly, the structure revealed a novel zinc-binding site in the amidohydrolase superfamily. The high-resolution structure should provide essential information regarding the diversity of the metal centres in the superfamily. Structural refinement based on a native data set at 1.5 Å resolution is still in progress and a full description of the refined structure will be published elsewhere.

Figure 2
The C^α trace of D-aminoacylase with an anomalous difference Fourier map contoured at 6σ showing 12 Se peaks. The smallest density peak is the tightly bound zinc ion.

We thank Drs N. Igarashi and N. Sakabe for kind help with data collection. The synchrotron-radiation work was supported by the Sakabe project at the TARA Center (Tsukuba Advanced Research Alliance), University of Tsukuba, Japan. This study was partly supported by Taiwan National Science Council Grant NSC 90-2321-B-002-002 and 90-2321-B-001-015.

References

Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–921.
Hendrickson, W. A., Horton, J. & LeMaster, D. M. (1990). *EMBO J.* **9**, 1665–1672.
Holm, L. & Sander, C. (1997). *Proteins*, **28**, 72–82.
Hsu, C. S., Lai, W. L., Chang, W. W., Liaw, S. H. & Tsai, Y. C. (2002). In the press.
Jones, T. A. & Kjeldgaard, M. (1997). *Methods Enzymol.* **277**, 173–208.
Kubo, K., Ishikura, T. & Fukagawa, Y. (1980). *J. Antibiot.* **33**, 556–565.
Leslie, A. G. W. (1997). *MOSFLM Users Guide: MOSFLM Version* 5.50. MRC Laboratory of Molecular Biology, Cambridge, England.
Moriguchi, M., Sakai, K., Miyamoto, Y. & Wakayama, M. (1993). *Biosci. Biotechnol. Biochem.* **57**, 1149–1152.
Perrakis, A., Morris, R. & Lamzin, V. S. (1999). *Nature Struct. Biol.* **6**, 458–463.
Sugie, M. & Suzuki, H. (1978). *Agric. Biol. Chem.* **44**, 107–113.
Tsai, Y. C., Lin, C. S., Tseng, T. H., Lee, H. & Wang, Y. J. (1992). *Enzyme Microb. Technol.* **14**, 384–389.
Wakayama, M., Ashika, T., Miyamoto, Y., Yoshikawa, T., Sonoda, Y., Sakai, K. & Moriguchi, M. (1995). *J. Biochem.* **118**, 204–209.
Wakayama, M., Katsuno, Y., Hayashi, S., Miyamoto, Y., Sakai, K. & Moriguchi, M. (1995). *Biosci. Biotechnol. Biochem.* **59**, 2115–2119.
Wakayama, M., Watanabe, E., Takenaka, Y., Miyamoto, Y., Tau, Y., Sakai, K. & Moriguchi, M. (1995). *J. Ferment. Bioeng.* **80**, 311–317.
Wakayama, M., Yada, H., Kanda, S., Hayashi, S., Yatsuda, Y., Sakai, K. & Moriguchi, M. (2000). *Biosci. Biotechnol. Biochem.* **64**, 1–8.
Watanabe, N., Nakagawa, A., Adachi, S. & Sakabe, N. (1995). *Rev. Sci. Instrum.* **66**, 1824–1826.
Yang, Y. B., Hsiao, K. M., Li, H., Yano, H., Tsugita, A. & Tsai, Y. C. (1992). *Biosci. Biotechnol. Biochem.* **56**, 1392–1395.
Yang, Y. B., Lin, C. S., Tseng, C. P., Wang, Y. J. & Tsai, Y. C. (1991). *Appl. Environ. Microbiol.* **57**, 1259–1260.