Structural determination of *Alcaligenes faecalis* DA1 D-aminoacylase by Se-SAD

Shen-Jia Chen[1], Chih-Yung Hu[1], Shwu-Huey Liaw[123]*
[1]Institute of Biochemistry, [2]Department of Life Science, National Yang-Ming University, and
[3]Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan

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. The D-aminoacylases are zinc enzymes, and removal of the bound zinc ions abolishes completely the catalytic activity. Recently, the putative metal chelating ligands were identified based on structural prediction and mutational studies. D-aminoacylases may belong to the recently identified amidohydrolase superfamily, in which only the metal ligands, four histidines and one aspartate, are strictly conserved. To understand the structural details of the metal-assistant catalysis, we have obtained the first D-aminoacylase crystal from *A. faecalis* DA1, and solved the structure by using a SeMet SAD data set at 1.8 Å resolution.

Materials and methods

The 483-residue *A. faecalis* DA1 D-aminoacylase was expressed in *E. coli* M15 and isolated by four chromatographic procedures as described previously [1-2]. To express the SeMet protein, the recombinant expression vector, the pQE30 (Qiagen), was transformed into *E. coli* B834 (DE3). The crystals suitable for the x-ray diffraction analysis were grown in 25-30% polyethylene glycol 4000, 0.1 M Na citrate (pH 5.6), and 0.2 M ammonium acetate. Data were collected at 100 K by using an ADSC Quantum 4 CCD camera at beamlines 6A and 18B of the Photon Factory of the High Energy Accelerator Research Organization, and then processed with the program *MOSFLM*. The phase problem was solved by using the program *CNS*. After heavy-atom search and density modification, the electron-density map was submitted to the automatic *wARP* procedure. The initial atomic model was subsequently refined by *CNS* and *TURBO*.

Results and discussion

Crystals could be obtained from the recombinant protein purified by either four columns or only the Ni-NTA (Qiagen) column. However, both the Ni-NTA and HiTrap Q (Pharmacia) columns were required for higher Se-Met protein purity suitable for crystallization. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 60.2 Å, b = 76.6 Å, and c = 135.3 Å. There is one molecule in an asymmetric unit (V_M = 2.9 Å^3/Da), with a solvent content of 57%. 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. 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. Twelve Se peaks were identified, and the first two methionines in the vector were disordered (Fig. 1). Unexpectedly, the structure revealed a novel zinc-binding site in the amidohydrolase 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 [3].

Table 1. Summary of data-collection statistics

Resolution (Å)	1.8 (1.9-1.8)
Total observations	360069
Unique reflect	56888
Completeness (%)	98.3 (94.4)
Anomalous completeness (%)	92.4 (80.7)
I/σ (I)	12.2
Multiplicity	
R_{merge} (%)	5.1 (15.7)
R_{anom} (%)	6.2 (11.0)

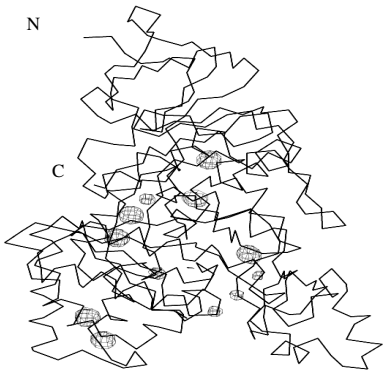

Fig. 1 The Cα trace of D-aminoacylase with an anomalous difference Fourier map contoured at 6 σ showing 12 Se peaks.

References

[1] C.-S. Hus et al., *Acta Cryst.* D. in press (2002).
[2] C.-S. Hus et al., in submission.
[3] S.-H. Liaw et al., in submission.

* shliaw@ym.edu.tw