ESRF	Experiment title: X-ray crystal structure analysis of l-aminocyclopropane- 1 -carboxylic acid deaminase	Experiment number: LS-856 LS-847
Beamline: BM14	Date of experiment: from: 24-Oct-97 to:26-Oct-97 8-Dec-97 to: 9-Dec-97	Date of report:
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Names and affiliations of applicants (* indicates experimentalists):

*Dr. Min Yao , Institute for Protein Research Osaka University, Japan
*Dr. Atsushi Nakagawa, Graduate School of Science Hokkaido Univ., Japan
*Mr. Hiroshi Sugimoto, Graduate School of Science Hokkaido Univ., Japan
Dr. Isao Tanaka, Graduate School of Science Hokkaido Univ., Japan
*Dr. Soichi Wakatsuki, ESRF, Grenoble, France

Report:

1. Abstract

A pyridoxal phosphate (PLP) -dependent enzyme, 1 -Aminocyclopropane- 1-carboxylic acid (ACC) deaminase found only in microorganisms catalyzes a reaction of cleavage ACC to α-ketobutyrate and ammonia by cyclopropane ring-opening. ACC is a key intermediate in the biosynthesis of a plant hormone ethylene which affects diverse growing and fruit ripening. Interestingly, although ACC deaminase has never been found in higher plants, introduction of ACC deaminase gene in plants was reported to reduce the production of ethylene and delay ripening progression of fruits.

The ACC deaminases from yeast *Hansenula saturnus* (yACCD) has been well characterized and also crystallized in earlier studies. The native crystal belongs to space group $C222_1$ with cell dimension of a=65.7Å, b=268.5Å, c=187.2Å. Two kinds of crystal forms for mercury derivative crystals were obtained by co-crystallization. One has similar cell to native crystals, and another one is the trigonal of $P3_221$(a=b=79.4Å, c=243.6Å and γ=120°). Since only one kind of non-isomorphous mercury derivative of was obtained, we decided to use the technique of multiple wavelength anomalous dispersion (MAD) rather than single isomorphous replacement with anomalous scattering (SIRAS). The beam-line MB14 in ESRF, Grenoble was advantageous owing to the high energy resolution to choose wavelengths for getting the maximum f'and f" anomalous scattering factor at edges of mercury atom.

2. Summary of the result obtained during the beam-time

During the first beam-time of the LS856, we collected the native data up to 2.0 Å resolution. However, there is not a enough time to collect a full MAD data set. At the next beam-time of the LS847*, based on the fluorescence spectrum of the mercury, atom, three 2.8 Å resolution data sets (pi, pk, rm) were collected using one co-crystal of mercury derivative of orthorhombic form with three wavelengths (1.0086: 1.0063, 0.9183) respectively. All data sets were obtained from frozen crystals at 100K with a detector of MAR345mm, and integrated by Denzo. Scaling and merging were calculated by SCALEPACK. The processing statistics are shown in Table.

Three of four independent mercury atoms were located from the Bijvoet anomalous difference Patterson maps of pi, pk data. The maximum-likelihood program SHARP was used for heavy-atom refinement and phasing following solvent flattening by the program SOLOMON using remote wavelength data as 'native' data The model was built based on 2.8 Å electron density map after improvement by non-crystallographic symmetry averaging of multiple crystal forms using the UPPSALA program package combined with SOLOMON and CCP4 program package with trigonal form data which were collected using synchrotron radiation at the Photon Factory of Japan and independently phased.

The molecular dynamic refinement is currently underway at 2.0 Å resolution using native data.

Table Summary of collected data

Resolution (A)	100 ~ 2.0	100 ~ *2.8*		
Wave length(A)	1.0	l.0086(pi)	l.0063(pk)	0.9183(rm)
Unquiet reflections	110,126	39,624	*40,440*	*38,708*
Completeness(%)	98.5	95.5	97.1	94.0
Averaged Redundancy	9.08	9.89	9.55	9.85
Averaged I / s (I)	14.6	11.1	11.9	*12.6*
R-merge (%)	5.1	*7.8 (6.9)*	*6.9 (5.9)*	*7.0 (6.1)*
R-remote (%)	26.9	*5.3*	*5.3*	

*The beam-time of LS847 was proposed for "Structure analysis of ribosomal protein S7" by the same group. Before the beam-time, the structure of ribosomal protein S7 had been solved using in-house search beam-time of BM14. Therefore, yACCD data collection was carried out in this beam-time,