The structure determination of yeast 1-aminocyclopropane-1-carboxylic acid deaminase by multiple wavelength anomalous dipersion method

Atsushi Nakagawa* (0003338), Min Yao (003056), Hiroshi Sugimoto (003340), Toyoyuki Ose (0003853), Atsushi Horiuchi, Isao Tanaka (0003339)
Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan

INTRODUCTION

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

Since only one useful mercury derivative of yACCD was found, we decided to solve the structure based on MAD method using the mercury atoms. MAD diffraction data collection was carried out on the beamline 41XU at the SPring-8. We could collect only one data set at one wavelength (λ=0.9Å). The Bijvoet anomalous difference Patterson map (Fig1) has shown prominent peaks corresponding to the two mercury atoms. The three-dimensional structure of yACCD has been solved using data collected afterwards. Here we report the structure of yACCD.

RESULTS

The two crystal forms were obtained for mercury derivative of yACCD by co-crystallization. The 2.0 Å native data and 2.8 Å MAD data of the orthorhombic form and 2.5 Å MAD data of the trigonal form were collected using synchrotron radiation at the ESRF of France and Photon Factory of Japan, respectively. All of data sets were collected in cold nitrogen gas stream at 100 K, and integrated by DENZO program. Initial phasing was done independently at 2.8 Å for the orthorhombic and 2.5 Å for the trigonal form by MAD data of mercury. Heavy-atom parameter refinement and phase calculation were carried out using the program SHARP. The model was built based on 2.8 Å electron density map after improvement by non-crystallographic symmetry averaging of multiple crystal forms. The refinement is currently underway at 2.0 Å resolution using native data.

The figure2 shows a view of the overall structure of yACCD for a monomer. The molecule consists of two domains with different size. The PLP is located at the site close to gap between two domains, and covalently bound to Lys51.

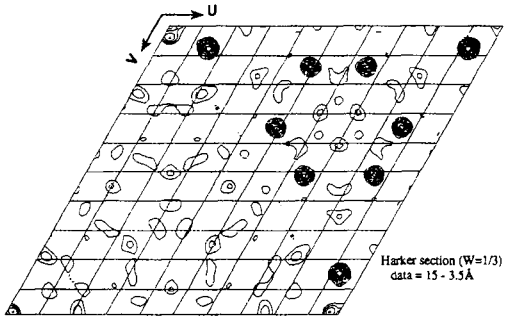

Fig1. The Bijvoet anomalous difference Patterson map of $P3_221$

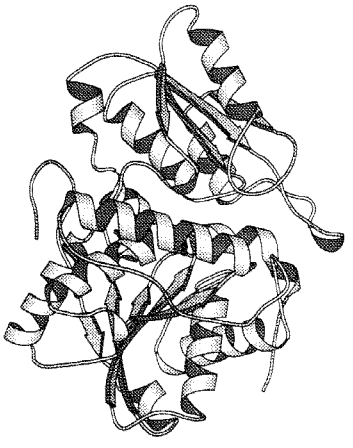

Fig2. A view of monomer structure of yACCD