

High Resolution Data Collection and Preliminary Laue Diffraction Study of Adenylate Kinase from *Sulfolobus solfataricus*

Hiroshi Yamaguchi (User Card Number : 0003475)*, Misturu Watanabe,
 Daisuke Kitaguchi†, Kaoru Mukumoto, Yukio Gotoh†, Tposhihide Okajima†
 School of Science, Kwansei Gakuin University,
 Faculty of Agriculture, Kinki University†

Archebacteria *Sulfolobus solfataricus* has been grown up at 75°C and pH3.0. Adenylate kinase from *Sulfolobus solfataricus* is a trimeric enzyme. Each subunit has a molecular weight of 21000. The enzyme catalyzes the transfer of a phosphoryl group from ATP to AMP according to:



The enzyme is stable at high temperature, and the enzymatic activities are slow down at low temperature. It is considered that the enzyme is suitable for a structural study by Laue method. We carried out X-ray diffraction study of the enzyme by using synchrotron radiation for two purposes. First; determining the high resolution structure to elucidate the role of thermostability. Second; examining the availability of crystals for Laue diffraction study to analyze the reaction mechanism.

High resolution data Collection

The crystals of adenylate kinase from *Sulfolobus solfataricus* are crystallized by using Ammonia sulfate as a precipitant at 20°C. Diffraction data were collected at the beamline BL44B2, SPring8, Hyogo, Japan. The crystals were transferred into the mother liquor containing 30 % glycerol as a

cryoprotectant. They were snared with the rayon loop and were placed directly in the nitrogen cooling stream of OXFORD CRYO-SYSTEM. The wavelength of 0.7 Å was used. Diffraction images were recorded on the RIGAKU R-AXIS IV imaging plates diffractometer, and were processed by using program PROCESS. The crystal belongs to trigonal system, the space group *R*32 with cell dimensions $a = 103.72 \text{ \AA}$, $c = 224.17 \text{ \AA}$ (hexagonal setting). A data set with 39067 unique reflections has been collected, giving a data set completeness of 94.7 % up to 1.8 Å resolution with an *R*merge of 6.35 %. Structure determination is in progress.

Laue Diffraction Study

The crystal was sealed in thin wall glass capillary. Ten frames of Laue diffraction image were recorded on R-AXIS IV diffractometer, at BL44B2. The crystal was exposed to white X-ray beam for 10 msec for each frame. The images of first seven frames were very well, while the images of the eighth, ninth and tenth frame were a little bit streaky. It is considered that the crystals are stable for irradiation of white X-ray beam. Preparation of complex crystal with cagedATP and substrates is in progress.