X-Ray Crystal Structure Analysis of *E. coli* Cysteine desulfrase 2

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Proteins with prosthetic groups of Fe-S cluster have been found to play numerous biological roles such as electron transport, catalysis, stabilization of protein structure, and regulation of metabolic pathways. In spite of the importance of these roles, little is known about how Fe-S clusters are formed *in vivo*.

Recently, it has been reported that there exist proteins such as NIFS participating in supply of sulfur in the synthesis of Fe-S clusters. The NIFS protein encoded by the *nif*S gene from a diazorotrophic bacterium *Azotobactor vinelandii* is involved in providing sulfur for the Fe-S clusters in the synthesis of nitrogenase. *Escherichia coli* has three genes similar to the *nif*S gene. These gene products resemble the NIFS protein not only in amino acid sequence but also in catalytic property. These NIFS-like proteins are novel PLP-dependent enzymes which contain pyridoxal 5'-phosphate as a coenzyme. Two of them decompose not only L-selenocysteine, L-selenocystine, L-cysteine, and L-cystine to L-alanine, and selenium atom or sulfur atom, like NIFS and the other NIFS-like protein of *E. coli*, but also cysteine sulfinic acid to L-alanine and sulfur dioxide. We have called them cysteine desulfrase 1 and 2 (CDS1 and CDS2), respectively. In order to study the structure-function relationship of the NIFS family of proteins by elucidating the reaction mechanisms of the *E. coli* NIFS-like proteins from their structural viewpoints, we have been carrying out X-ray structure analysis of CDS2 which has first been crystallized.

CDS2 crystals were obtained at 25℃ by hanging-drop vapor diffusion against sodium acetate (pH6.8). The crystal shape is a tetragonal bipyramide with typical dimensions of 0.4 x 0.3 x 0.3 mm. The crystals belong to tetragonal space group $P4_32_12$ with cell dimensions a=b=128.1 Å and c=137.0 Å.

For a detailed discussion on the reaction mechanism of enzyme based on an accurate determination of high-resolution stereo-structure of the enzyme, we carried out synchrotron experiments of the present enzyme crystal on the beamline BL41XU of Spring-8. Several sets of diffraction patterns from the native crystals were recorded on imaging plates using monochromatized X-ray with wavelength of 0.708 Å. Each crystal was mounted with the crystallographic c-axis parallel to the rotation axis. Before each data collection, two shots of 1.0° oscillation were taken for indexing. In the data collection, the oscillation angle and exposure time per frame were 5.0° and 15 sec, respectively. The independent range of each crystal was covered by 10 frames. The visuallization of these diffraction patterns has revealed the gradual death of crystal because of radiation damage. This result strongly suggests the necessity of cryocrystallography in SPring-8. Usuable sets of diffraction patterns are now under process.