Recombinant expression and crystallographic analysis of thiocyanate hydrolase from *Thiobacillus thioparus*

Takatoshi Arakawa[1], Shingo Kataoka[1], Yoshiaki Kawano[2], Nobuo Kamiya[2], Yoko Katatyama[3], Masafumi Yohda[1], and Masafumi Odaka[1]

[1]*Faculty of Biotechnology and Life Science, Graduate School of Technology, Tokyo University of Agriculture and Technology*, [2]*RIKEN Harima Institute/Spring-8* , [3]*Faculty of Agriculture, Tokyo University of Agriculture and Technology*

Thiocyanate hydrolase (SCNase) of *Thiobacillus thioparus* THI115 is a dodecameric Co^{3+} -containing enzyme with the subunit stoichiometry of $\alpha:\beta:\gamma=1:1:1$. It catalyzes the degradation of thiocyanate to carbonyl sulfide and ammonia. SCNase subunits are highly homologous to nitrile hydratases (NHases) in amino acid sequences. Like NHase, one of the cysteine residues presumably coordinating to the metal ions is post-translationally modified to cysteine-sulfinic acid (Cys-SO_2H). Here, we report the recombinant expression of SCNases as apo- and holo-forms in E. coli and X-ray crystal structure analyses of the native as well as apo-SCNases.

The apo-SCNase was expressed in E. coli transformed with a vector harboring with the gens of α–, β– and γ subunits. In contrast, when SCNase subunit genes were co-expressed with the open reading frame adjacent to SCNase subunits genes, *orf1*, about 40 % of expressed SCNase was as the holo-form. This result suggested that ORF1, designated as P15k, is responsible for metal insertion and/or formation of the cysteine post-translational modifications.

On crystallization, recombinant apo-SCNase was purified and concentrated to 17mg/mL. Crystals were grown by hanging vapor diffusion technique in several conditions. X-ray diffractometric experiments were carried at Photon Factory and SPring-8. The crystals belonged to primitive tetragonal and primitive orthorhombic space group, respectively. The phases were determined by the multiple-wavelength anomalous dispersion (MAD) methods using the crystals soaked with heavy metals. The initial model structure was well interpretable form the obtained electron density maps. The iterative energy minimizations and thermal factor corrections were performed for the maps. We also succeeded in the crystallization of native SCNase with the same experimental condition. The solution structure of apo-SCNase was used as the model structure to determine holo-SCNase by molecular replacement method. Refinement of holo-SCNase is now in progress.