Dynamic Structures and Reaction Mechanism of Active Fe-type Nitrile Hydratase

Yoshiaki Kawano, Kouichi Hashimoto, Nobuo Kamiya, *Advanced Protein Crystallography Research Group, RIKEN Harima Institute / SPring-8, Japan.* E-mail:ykawano@spring8.or.jp

Nitrile hydratase from *Rodococcus* sp. N-771 is the enzyme that catalyzes the hydration of nitriles to the corresponding amides, and contains a mononuclear non-heme iron as the reaction center (Fe-type NHase). The center is photo-reactive, inactivated by nitrosylation and activated by photo-driven NO release. The photo-activated Fe-type NHase loses the activity within 24 hours under aerobic conditions. Previous studies have revealed that the post-translationally modified cystein sulfenate (αCys114-SO^-) of active enzyme is further oxidized under the aerobic conditions to cystein sulfinate (αCys114-SO_2^-).

In order to avoid the further oxidation, a crystallization system was constructed under anaerobic conditions of less than 0.1% (v/v) oxygen concentration. The really active structure of intact Fe-type NHase was studied by X-ray crystallography, including complex structures with butyric acid as an inhibitor/stabilizer and with cyclohexyl-isocyanide (ch-NC) as a substrate analogue. We also crystallized the inactive nitrosylated NHase under the anaerobic conditions in the complex form with ch-NC, and dynamic structure changes were observed after photo-activation at a time-resolution of 30min using the large-angle oscillation technique (LOT) at a RIKEN beamline: BL45XU, SPring-8. Based on the results obtained, we will discuss the role of αCys114-SO^- in the nitrile hydration mechanism of Fe-type NHase.

Keywords: nitrile hydratase, cysteine sulfenate, dynamic structure