

[P4] QM/MM Study of Catalytic Mechanism of Nitrile Hydratase

Megumi Kayanuma¹, Mitsuo Shoji², Yasuteru Shigeta²

¹Graduate School of Systems and Information Engineering, University of Tsukuba, 1-1-1 Tennodai, 305-8573, Tsukuba, Ibaraki, Japan.

²Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, 305-8573, Tsukuba, Ibaraki, Japan.

Nitrile Hydratase (NHase) is a microbial enzyme which catalyzes hydration of nitriles to the corresponding amides and has been used for industrial productions of various amides such as an acrylamide. At the active site of NHase, a Fe(III) or Co(III) ion is located, and the coordination structure of the ion is quite unique in which contains two carboxamido nitrogens, one cysteine-sulfur (Cys-S), one cysteine-sulfenic (Cys-SO) and one cysteine-sulfinic (Cys-SO₂) moieties. The catalytic mechanism of this enzyme has not yet been elucidated though several paths have been proposed. Recently, Hopmann et al. have analyzed several reaction paths using quantum chemical calculations using an active-site model of a Fe-containing NHase [1-3]. However, there has been no theoretical analysis of the reaction mechanism of NHase considering the effects of the protein environment.

In the present study, we examined four reaction mechanisms (figure 1) by using Quantum Mechanics/ Molecular Mechanics (QM/MM) method. The QM region (figure 2) was calculated using DFT with B3LYP functional and LANL2DZ basis sets for iron atom and 6-31G(d,p) basis sets for the others. The MM atoms were subjected to the AMBER-99 force field. The QM/MM calculations were performed using the NWChem program package. Our calculations revealed that the barrier height of the C-O bond formation step in the path (a) is 10.3 kcal/mol, though the Fe-substrate coordinated structure is less stable than the Fe-water structures by more than 10 kcal/mol in electronic energy. The Fe-water structure is stabilized due both to ligand coordination and to hydrogen-bond network of β Tyr37- β Tyr72- α Ser113-water- α Cys112/ α Cys114- β Arg56/ β Arg141. The barrier height of the path (b) is too high (34.9 kcal/mol). In the path (c), the intermediate structure (nitrile carbon of the substrate and sulfenic oxygen of α Cys114 are bound) might be prevented for nitriles with larger substituents because of a steric hindrance of surrounding residues around α Cys114 (α Gln90 and β Arg56) so that we can rule out it.

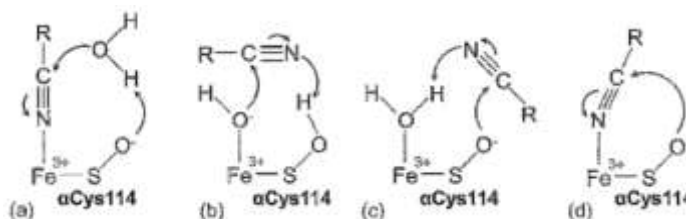


Figure 1 Four reaction mechanisms analyzed in the present study

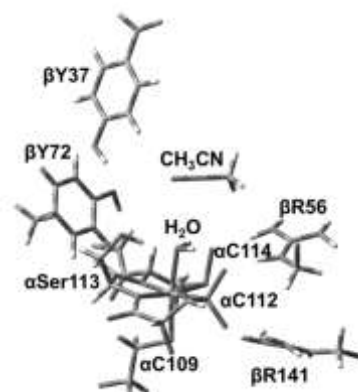


Figure 2 QM region

Bibliography:

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