Mutational study on Fe-type nitrile hydratase from *Rhodococcus* sp. N771 –the hydrogen bond network between αGln90 and αCys114 is responsible for the catalytic reaction–

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Nitrile hydratase (NHase) from *Rhodococcus* sp. N-771 is a non-heme iron enzyme having two post-translationally modified cysteine ligands, αCys112-SO_2H and αCys114-SOH at the catalytic center. The crystal structure of NHase in the nitrosylated form suggested the importance of hydrogen-bond networks in stabilizing the catalytic center structure. αGln90 was strictly conserved in all known NHases and involved in hydrogen-bond network around the catalytic center. In this study, we replaced αGln90, which is hydrogen bonded with both αSer113 and αCys114-SOH via hydration water molecules by glutamic acid or asparagines, and characterized biochemically. The *k*cat values of αQ90E and αQ90N mutant NHases decreased to approximately 24% and 5% that of wild-type, respectively but the effect of mutations on *K*m was not so significant. Both mutants conserved the two cysteine modifications. In both mutants, the αCys114-SOH modification was suggested to be responsible for the catalysis like native NHase. We elucidated the crystal structure of αQ90N mutant in the nitrosylated state at 1.43 Å resolution. The structure is basically identical to that of native nitrosylated NHase except for the mutated site and its vicinity structure. The structural difference between native and αQ90N mutant NHases pointed out the hydrogen bond network between αGln90 and αCys114-SOH is important for the catalytic activity.