Mechanistic Studies on the Nitrile Hydratase from *Pseudonocardia thermophila* JCM 3095.

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ABSTRACT. Nitrile hydratases (NHases) catalyze the hydration of nitriles to their corresponding amides. These biocatalysts have several potential applications including synthetic chemistry and bioremediation. Currently nitrile hydratases are used in the industrial production of acrylamide and nicotinamide from the corresponding nitriles. NHase is a metalloenzyme containing either a low spin non-heme Fe (III) or a low-spin non-corrin Co (III) ion in the catalytic center, designated as Fe-type and Co-type NHase families. NHases consist of α and β subunits, the amino acid sequences of which do not exhibit homology, and are typically $\alpha_2\beta_2$ heterotetramers. In all known NHases, each subunit has a highly homologous amino acid sequence (CXYCSCX) that forms the metal binding site. Fe-type NHase shows photoreactivity and binds a nitric oxide molecule, whereas Co-type NHase dose not. Fe-type NHase preferentially hydrates small aliphatic nitriles, whereas Co-type NHase exhibits a high affinity for aromatic nitriles. The NHase from *Pseudonocardia thermophila* JCM 3095 is Co-type NHase that has been over expressed in *Escherichia coli* and has been crystallographically characterized. Although the structures of Fe-type NHase and Co-type NHase are very similar and exhibit high sequence homology, Fe-type NHases only bind Fe (III) and Co-type NHases only bind Co(III). It is unknown at this time why NHases specifically select cobalt or iron. Moreover, the molecular characterization of both Fe-type and Co-type NHase enzymes has provided some insight into how the molecular structure controls the enzyme function. Based on these data and several elegant studies on active site NHase model complexes, four possible reaction mechanisms have been proposed. In order to determine which proposed reaction mechanism is utilized by NHases, we have begun to kinetically characterize the NHase from *Pseudonocardia thermophila*. We are also investigating the catalytic efficiency of alternate substrates as well as the binding of potential substrate and transition-state analog inhibitors.