Synthetic Models of Nitrile Hydratase

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Nitrile Hydratase (NHase) is a hydrolytic enzyme that incorporates either a non-heme iron(III) or non-corrin cobalt(III) for the catalytic conversion of nitriles to amides.[1] The enzyme active site possesses some remarkable properties, among which are an unusual role for a common metal, a novel NO-regulatory mechanism, and unprecedented coordination sphere. The metal resides in an N_2S_3 donor environment comprised of two deprotonated amides from the protein backbone and three cysteine sulfurs. X-ray structural characterization of the NO-inactivated form of NHase from *Rhodococcus* sp. N-771 at 1.7 Å resolution indicated two of the cysteines had been post-translationally modified resulting in a mixed thiolato (RS^-), sulfenato ($RS(O)^-$), sulfinato (RSO_2^-) donor set.[2] Sulfur oxygenation was subsequently shown to be required for enzymatic activity although the role it serves remains undetermined.[3]

Recently, our group has reported the synthesis of a series of model complexes of iron-containing nitrile hydratase based on the penta-coordinate, dithiolato ligand 4,7-bis(2-methyl-2mercaptopropyl)-1-thia-4,7-diazacyclononane (bmmp-TASN, H_2L^1), where the sixth coordination site remains variable. Through careful choice of ligand, we have shown that the spin state of the iron atom is controllable. With the goal of substituting weakly donating ligands at the sixth position, we have undertaken the synthesis of a five coordinate L1Fe complex with a non-coordinating counter ion in hopes of facilitating our aims. L^1FeX ($X=F_3CSO_3^-$, PF_6^-) displays solvent dependent coordination behavior in THF and acetonitrile, with an apparent variability in the metal ion spin-state. Efforts to fully characterize this behavior are currently under way.

1.Kovacs, J.A. *Chem. Rev*. **2004**, 104(2), 825-848; Harrop, T.C.; Mascharak, P.K. *Acc. Chem. Res*. **2004**, 37(4), 253-260. 2. Nagashima, S.; Nakasako, M.; Dohmae, N.; Tsujimura, M.; Tokoi, K.; Odaka, M.; Yohda, M.; Kamiya, N.; Endo, I. *Nat. Struct. Biol*. **1998**, 5, 347-351. 3. Murakami, T.; Nojiri, M.; Nakayama, H.; Odaka, M.; Yodha, M.; Dohmae, N.; Takio, K.; Nagamune, T.; Endo, I. *Protein Sci.* **2000**, 9, 1024-1030. 4. Grapperhaus, C.A.; Li, M.; Patra, A.K.; Poturovic, S.; Kozlowski, P.M.; Zgierski, M.Z.; Mashuta, M.S. *Inorg. Chem*. **2003**, 42(14), 4382-4388.