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Solution Structure of Quasi-Stable Nitrile Hydratase Model Compounds Probed by Fe-K edge XAS.

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Beamline(s): X9B

Introduction: Nitrile Hydratases (NHases) are hydrolytic metalloenzymes that convert nitriles to amides and contain a non-heme iron in its active-site that is ligated by three cysteinyl sulfurs, two amide nitrogens, and a water (or hydroxide) molecule.[1] A possible mechanism of nitrile hydrolysis involves the displacement of water from the metal-center by a nitrile, leading to CN bond activation, followed by water (or hydroxide) attack. To test the validity of such a scenario, we have prepared model complexes that weakly bind nitriles and methanol (Figure 1a [Fe^{III}](PF₆)⁻ (**1**)). Solution Fe-K edge XAS was used to determine if these models do indeed bind a nitrile or methanol.

Methods and Materials: 25 mg of **1** was dissolved in either CH₂Cl₂, MeOH, or MeCN, injected between two pieces of 1 mil 3M Kapton tape sealing an aluminum sample holder, and quickly frozen in liquid nitrogen. XAFS data was collected in fluorescence mode on beam line X9B at 77 K. Data analysis and graphing employed "RCSEXAFSPackage" written for *Igor Pro* software and general methods that have been described previously.[2]

Results: The 1s→3d transition for **1** dissolved in all three solvents found in the XANES spectra is depicted in Figure 1b. As can be seen, a decrease in intensity of this transition is observed going from the non-coordinating solvent CH₂Cl₂ to the coordinating solvent MeCN and MeOH. This is consistent with a change in coordination environment from a less to a more centrosymmetric molecule (i.e. a five- to a six-coordinate complex), and implies nitrile and methanol coordination. This change in coordination environment was supported by analysis of the EXAFS region (Figure 1c). Complex **1** dissolved in CH₂Cl₂ supports a 3-nitrogen 2-sulfur coordination environment with average Fe-N bond-lengths of 2.000(18) Å and average Fe-S bond-lengths of 2.121(1) Å. These bond-lengths are consistent with the S = 1/2 spin-state of the complex. Addition of MeOH or MeCN leads to a lengthening of the average Fe-N and Fe-S bond lengths, consistent with coordination of a sixth ligand. Addition of a sixth ligand (oxygen or nitrogen scatterer) is also required for an accurate solution of the EXAFS.

Conclusions: XANES and EXAFS spectroscopy carried out at the NSLS that NHase model complexes can ligate the neutral ligand MeCN and MeOH. This work has recently been published. [2]

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References:

[1] Kobayashi, M. *Nat. Biotech.* **1998**, *16*, 733 - 736.

[2] Shearer, J.; Jackson, H. L.; Schweitzer, D.; Rittenberg, D. K.; Leavy, T. M.; Kaminsky, W.; Scarrow, R. C.; Kovacs, J. A. *J. Am. Chem. Soc.* **2002**, *124*, 11417 – 11428.

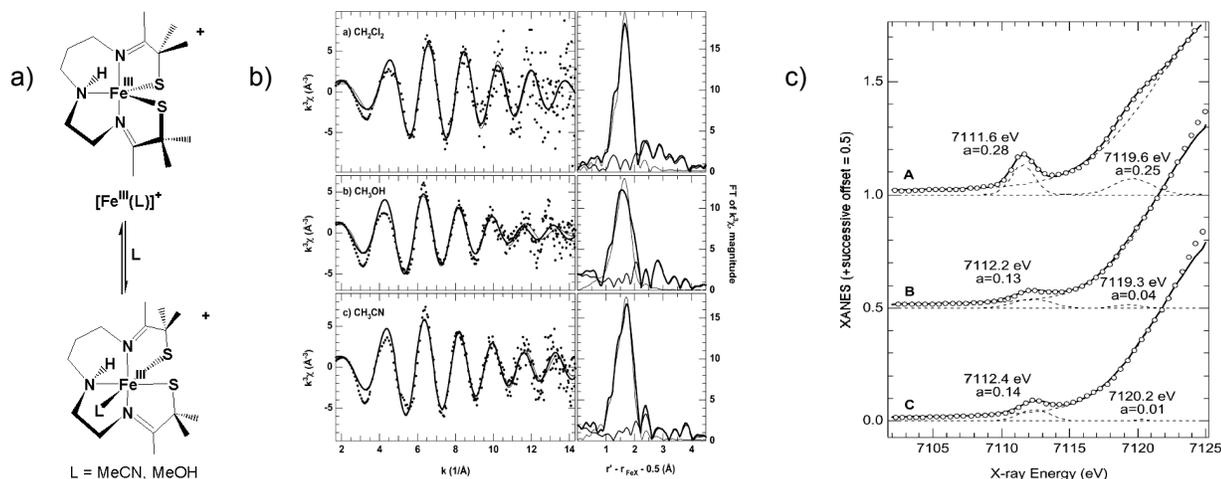


Figure: (a) Reaction scheme for formation of NHase model complexes discussed in this abstract. (b) Comparison of XANES spectra of complexes (A= CH₂Cl₂, B = MeOH, C = MeCN). (c) Comparison of Fourier Transforms and Unfiltered EXAFS ($k^3(\chi)$) for the complexes in CH₂Cl₂, MeOH, and MeCN.