Understanding the Effect of Single Oxygen Atom Addition on the Properties of an Fe-Nitrile Hydratase Analogue

Priscilla Lugo-Mas[1], Liang Xu[1], Steven D. Davin[1], Abhishek Dey[2], Jason Benedict[1], Werner Kaminsky[1], Edward I. Solomon[2], and Julie A. Kovacs[1]

[1]Department of Chemistry, University of Washington, [2]Department of Chemistry, Stanford University

Nitrile hydratase (NHase) is a cysteinate–ligated non–heme iron enzyme involved in microbial nitrile hydrolysis. An unusual aspect of NHase is that two of the three coordinated cysteinate sulfurs appear to be oxidized (post-translationally modified)- one to a sulfenate (^{114}Cys-S=(O)) or sulfenic acid (^{114}Cys-S-(OH)), and the other to a sulfinate (^{112}Cys-S=$(O)_2$) or sulfinic acid (^{112}Cys-SO_2H). It has yet to be proven definitively whether both of these oxidized cysteinates are required for catalytic activity. Recently it was suggested that the sulfenate oxygen may be intimately involved in the NHase mechanism. In order to understand how the addition of a single oxygen atom would perturb the properties of a thiolate-ligated Fe-NHase analogue, we report herein the synthesis of the first example of a sulfenate-ligated Fe-NHase model complex, $[Fe^{III}(ADIT)(ADIT\text{-}O)]^+$ (**1**), and examine its electronic, magnetic, and redox properties, and study its reactivity towards Bronsted and Lewis acids. Despite the dramatic changes and the unexpected blue-shift in the electronic absorption spectrum, addition of a single oxygen atom to $[Fe^{III}(ADIT)_2]^+$ (**2**) does not dramatically affect its redox and magnetic properties. Reversible protonation of the sulfenate oxygen of **1** occurs upon addition of strong acids at low temperatures as evidenced by a red-shift in the LMCT band (without loss of intensity). Alternatively, addition of strong acids to **2** results in a blue-shift of the LMCT band (without loss of intensity) suggesting a different protonation site. Zinc binding at the sulfenate oxygen is demonstrated by an X-ray structure of $[Fe^{III}(ADIT)(ADIT\text{-}O\text{-}ZnCl_3)]$ (**3**). Oxygen–atom abstraction from **1** occurs with PEt_3, albeit under forcing conditions. Sulfur K-edge XAS data as well as DFT calculations show that these changes to the geometric and electronic structure of **1** involve the thiolate sulfur compensating for the removal of electron density from the metal ion (via the S(R)–O bond) by increasing its π–back donation to the metal. The implications of these results with respect to the function of the NHase cysteine sulfenate will be discussed.