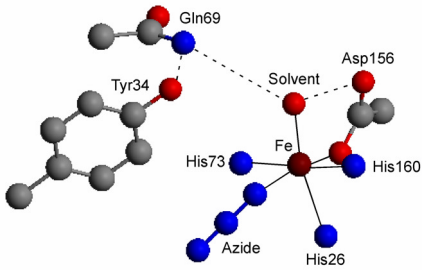
Spectroscopic and Computational Studies on Substrate Analogue Interactions with Iron(III) Superoxide Dismutase

Laurie E. Grove[1], Juan Xie[1], Emine Yikilmaz[2], Anne-Frances Miller[2], Jason A. Halfen[3], and Thomas C. Brunold[1]

[1]Department of Chemistry, University of Wisconsin-Madison, [2]Department of Chemistry, University of Kentucky, and [3]Department of Chemistry, University of Wisconsin-Eau Claire

Iron superoxide dismutase (FeSOD) and manganese superoxide dismutase (MnSOD) belong to a family of enzymes responsible for the protection of aerobic organisms against the superoxide radical anion, $O_2^{\cdot-}$, by catalyzing the disproportionation of $O_2^{\cdot-}$ to O_2 and H_2O_2. Interestingly, these two enzymes are highly homologous in terms of both their protein folds and their active sites structures; however, both enzymes are highly metal-specific and lose activity upon incorporation of the non-native metal (e.g. Fe-substituted MnSOD, Fe(Mn)SOD). This metal specificity has been attributed to the precise redox tuning of the active site metal ion through hydrogen bonding interactions involving second-sphere residues. To assess the role these second-sphere residues have in catalysis, it is necessary to understand the mechanism for each enzyme. However, the turnover rates for these enzymes approach the diffusion-controlled limit and thus inhibitors with similar size and frontier orbitals, such as azide, have been used to evaluate possible reaction mechanisms using both spectroscopic and computational tools.



Active site of azide-bound Fe^{3+}SOD

To resolve the current ambiguity of whether two azides can concurrently bind to Fe(III) in FeSOD by displacing the solvent molecule (see Figure), we have synthesized a di-azide Fe(III) model complex and used magnetic circular dichroism (MCD) and resonance Raman (rR) spectroscopies to identify the spectral features inherent to such a species. Additionally, to explore the role of second-sphere residues in tuning the active site properties, we have performed quantum mechanics (QM) and combined quantum mechanics/molecular mechanics (QM/MM) calculations to generate spectroscopically validated models for the azide-bound forms of FeSOD, Fe(Mn)SOD, and the Q69E FeSOD mutant. These models provide significant insight into how the second-sphere residues modulate the reduction midpoint potential of the active-site metal ion.