Manganese or Iron? Spectroscopic Studies on the Inorganic Cofactor of the *C. ammoniagenes* Ribonucleotide Reductase.

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Most ribonucleotide reductases (RNR) activate dioxygen with a diferrous cluster to oxidize a tyrosine residue to a catalytic tyrosyl radical and a diferric cluster cofactor. In RNR from Coryneform bacteria, the release of Mn into solution when active, purified enzyme is acid denatured implicates a Mn cofactor catalyzing the analogous reaction. This manganese dependence has recently been questioned, however, based on several lines of evidence. Gene sequence homology to iron forms of the enzyme and observation of higher activity and tyrosyl radical formation when iron is bound to overexpressed enzyme suggest that the *C. ammoniagenes* RNR is a Class 1b Fe enzyme. Recently, the crystal structure of the Mn-bound form of the *C. ammoniagenes* RNR implies that the Mn in the enzyme is reduced and therefore catalytically inactive.

We have also been addressing the question of the metal specificity of the RNR from *C. ammoniagenes.* When our overexpressed enzyme is exposed to iron, we observe the classic spectroscopic signatures of oxygen activation at the iron center, such as the EPR signal of the tyrosyl radical and the UV/Vis absorbances of both a diferric cluster and tyrosyl radical. However, when our as-isolated enzyme is exposed to manganese, we observe tyrosyl radical formation and the corresponding UV/Vis signature of a Mn(III) dimer similar to those observed for Mn(III) dimeric model complexes and for the Mn catalases. Furthermore, we measured the Mn X-ray absorption edge of our *C. ammoniagenes* Mn enzyme after exposure to oxygen and observed both a change in shape and a small increase in edge energy relative to the edge of an inert Mn(II) form of the *E. coli* Class 1a enzyme. In addition, the EXAFS spectra of the Mn *C. ammoniagenes* and *E. coli* enzymes are also consistent with Mn oxidation in the former. Taken together, the evidence presented here suggests that a manganese cluster in the RNR from *C. ammoniagenes* can activate oxygen in order to generate a tyrosyl radical.