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# Spectroscopic Studies of Heme Proteins/Enzymes Involved in Small Molecule Sensing/Activation

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Hemes are among the most widespread and important cofactors in biochemistry. Best known among the heme proteins are the oxygen transport and storage proteins, respectively hemoglobin and myoglobin. The best known among the heme enzymes is the heme oxidase cytochrome P450 (CYP), found throughout living systems including a vital role in human metabolism of xenobiotics.[1] There are, however, many other heme enzymes/proteins of interest besides these "paradigm" examples, in particular other hemes with proximal thiolate coordination.[2] These include the carbon monoxide (CO) sensing protein CooA,[3] and the nitric oxide (NO)-dependent nitrating enzyme TxtE, which is a cytochrome P450 homologue.[4] Ferric heme, i.e., porphyrin with Fe(III) $3d^5$, can be in the high-spin ($S$ = 5/2) or low-spin ($S$ = 1/2) ground state. In either spin state, electron paramagnetic resonance (EPR) spectroscopy can provide valuable information on electronic structure and substrate/product/inhibitor binding. Specialized forms of EPR, such as electron nuclear double resonance (ENDOR) and electron spin echo envelope modulation (ESEEM) spectroscopies can provide even more information, specifically about nuclei that comprise the active site environment.[5] We describe EPR and ENDOR studies on both CooA and TxtE, including parallel comparative studies on a P450 (CYP119) enzyme. All three of these systems contain heme with proximal cysteine thiolate coordination, yet the electronic effect of this ligand differs in each case. In the case of CooA, in collaboration with the Burstyn group, a series of single point mutants probes the effects of H-bonding on structure. In the case of TxtE, in collaboration with the Caranto group, only wild type has been investigated thus far, but the effect of substrate (L-tryptophan) and analogs is explored.

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