

Elucidation of Ligand Hyperfine Couplings for Multi-Copper Oxidases Using Two-Dimensional ESEEM Spectroscopy

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Four pulse Hyperfine Sublevel Correlation Spectroscopy (HYSCORE), a two-dimensional electron spin echo envelope modulation (ESEEM) technique, was used to resolve ligand hyperfine couplings for the type 1 and type 2 Cu(II) sites of Fet3p from *Saccharomyces cerevisiae* and *Rhus vernicifera* laccase. For studies of histidyl imidazole coordination, HYSCORE cross-peaks provide information on hyperfine frequency correlations that are necessary for a systematic determination of hyperfine tensor elements and ^{14}N nuclear quadrupole coupling parameters. For the Fet3p protein, HYSCORE clearly distinguishes contributions from type 1 and type 2 coppers, circumventing a problem that has prevented a rigorous analysis of histidyl imidazole bonding to the type 1 Cu(II). Using these new data together with information derived from model compound studies, we find that there are two histidyl ligands coordinated to the type 1 Cu(II) of Fet3p and that these two ligands differ in terms of hyperfine coupling strength, the orientation of the imidazole plane with respect to the magnetic axes of the metal ion, and the symmetry of the remote nitrogen nuclear quadrupole interaction. These results will be discussed in terms of recent findings regarding the structure and mechanism of substrate Fe(II) oxidation.¹ A comparison of our Fet3p results with similar studies done on *Rhus vernicifera* laccase will also be presented.

¹ Qunitanar, L.; Gebhard, M.; Wang, T-P; Kosman, D.J. and Solomon, E.I. *J. Am. Chem. Soc.* 2004, **126**, 6579-6589.