Pulsed EPR Studies of ^{17}O-Labeled Sulfite Oxidase and Model Compounds

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Sulfite oxidizing enzymes are molybdenum-containing proteins which play a vital role in the sulfur metabolism of plants, animals and bacteria by catalyzing the two-electron oxidation of sulfite to sulfate. During turnover, the molybdenum center passes through a Mo(V) state, which can be studied using electron paramagnetic resonance (EPR) spectroscopy. The structure of the active site can be determined from the hyperfine and quadrupole couplings of magnetic nuclei in the vicinity of the Mo ion. Our recent pulsed EPR experiments have focused on incubating samples of chicken sulfite oxidase with ^{17}O water (I = 5/2) in order to follow the trafficking of oxygen atoms through the enzyme's catalytic cycle. Electron spin echo envelope modulation (ESEEM) experiments have indicated the presence of a weakly magnetically coupled (hyperfine interaction constant ~ 5 MHz) oxygen ligand that has been assigned to the Mo-oxo group. The validity of this assignment was confirmed through ESEEM examination of a ^{17}O-labeled model compound, $[Mo^{17}O(SPh)_4]^-$. In the model compound, two-pulse ESEEM spectra revealed the ^{17}O isotropic hyperfine coupling constant to be about 6.5 MHz, while a quadrupole coupling constant on the order of 1 MHz was determined from the four-pulse ESEEM spectra. These results support the original assignment of the weakly magnetically coupled oxygen in sulfite oxidase to the Mo=O group, and also provide information that can be used to interpret the more complex protein spectra.