HIGH-FIELD ELECTRON PARAMAGNETIC RESONANCE OF IRON SUPEROXIDE DISMUTASE

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Introduction

Over the past several years, we have investigated the electronic structure of high-spin (HS), transition metal ion complexes by high–frequency and –field electron paramagnetic resonance (HFEPR). These efforts have led to successful studies of a variety of small molecules, including complexes of high-spin Fe(II)\(^{1,2}\). An experimentally more difficult challenge is to use HFEPR to study transition metal ions in metalloproteins. Such biological systems must be studied in solution, in a medium of frozen aqueous buffer and necessarily at concentrations much lower than that for small molecules.

A metalloprotein that we have chosen for initial HFEPR studies is iron superoxide dismutase, FeSOD, which catalyzes the physiologically important disproportionation of superoxide ion into dioxygen and hydrogen peroxide\(^{3}\):

\[
2 \text{O}_2^* + 2 \text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2
\]

FeSOD has been studied by other techniques, including magnetic circular dichroism\(^{4}\), and conventional EPR\(^{5}\). The active site consists of an Fe(III) ion coordinated by three histidine nitrogen atoms, an asparate oxygen atom, and a water/hydroxide oxygen atom (which can be replaced by exogenous ligands), in a trigonal bipyramidal geometry\(^{3}\), with an \(S = 5/2\) ground state. The extensive number of site-directed mutants of FeSOD\(^{6}\) will allow HFEPR to probe the relation between the electronic properties of the Fe(III) ion and biochemical structure and activity of the enzyme.

Experimental

E. coli FeSOD was isolated and prepared as described previously\(^{4,6}\). HFEPR spectra were recorded at NMHFL EMR facility using a superconducting magnet.

Results and Discussion

Figure 1 shows HFEPR spectra of wild-type FeSOD at ca. 95 and 184 GHz GHz and 10 K. The spectra contain many features, some of which may be attributed to high-spin Fe(III). The \(g \sim 2\) feature in both traces can be resolved in high-resolution conditions and has its origin in Mn(II) in a low-symmetry environment. Note that in addition to FeSOD, certain organisms produce MnSOD proteins\(^{3}\).

A full analysis of the multifrequency data set is currently in progress. The results will be combined with those from other techniques to yield a ligand-field description of the ferric ion in FeSOD.

References