Parallel Polarization EPR Characterization of the Mn(III) Center of Oxidized Manganese Superoxide Dismutase

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Manganese superoxide dismutase (MnSOD) is an enzyme found in mitochondria and chloroplasts of eukaryotes and in the cytoplasm of bacteria. The redox active Mn ion cofactor catalyzes the one-electron redox cycle by a two-step disproportionation reaction with oxidized Mn$^{3+}$ in the resting state.

In this report, we have used parallel polarization CW-EPR to investigate the paramagnetic Mn$^{3+}$ ion of the MnSOD enzyme from Escherichia coli overexpressed from pALS1 in HMS174/DE3. The crystal structure of native MnSOD from E. coli has been determined to 2.1 Å resolution. Crystal structures have also been solved for MnSOD of Thermus thermophilus and human mitochondria. These structures show high homology between the bacterial and the eukaryotic MnSOD and confirm that in each case the Mn$^{3+}$ ion has five ligands (three histidines, one aspartate, and one hydroxide ion) with a distorted trigonal–bipyramidal geometry. In this configuration, the d$^{4}$ Mn$^{3+}$ ion is high-spin with an effective total spin $S = 2$.\(^{6,7}\)

The energy levels of an $S = 2$ integer spin system with a positive axial zero-field splitting value, $D$, are arranged as shown in Figure 1. An EPR signal from the MnSOD Mn$^{3+}$ ion has not previously been detected with conventional X-band EPR methods. $D$ is predicted to be more than 2 cm$^{-1}$ based on SQUID saturation magnetization studies of the oxidized form of native MnSOD,\(^{7}\) or between 1–2 cm$^{-1}$ based on MCD studies.\(^{6,8}\) Because of the large zero-field splitting values, the conventional perpendicular polarization EPR allowed $\Delta M_s = \pm 1$ transitions are not possible at normal X-band microwave frequencies (0.3 cm$^{-1}$) (See Figure 1). However, EPR signals from integer spin systems can be detected when the oscillating magnetic field applied to induce a spin-state transition is oriented parallel to the static magnetic field. The parallel magnetic field orientation allows transitions between the closely spaced $M_s = \pm 2$ energy levels (Figure 1 inset), to be observed with signal intensities many orders of magnitude larger than the corresponding perpendicular magnetic field orientation.\(^{8}\)

Figure 2a shows the parallel polarization EPR spectrum of the Mn$^{3+}$ ion of native MnSOD. This signal is centered at an effective

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Figure 1. Energy level diagram of an $S = 2$ spin system, obtained via eq 1 with matrix diagonalization, with $D = 2.10$ cm$^{-1}$, $E = 0.243$ cm$^{-1}$, $A_s = A_t = 101$ G, $A_i = 100$ G, $g_s = 2.00$, $g_i = 1.99$, $g_t = 1.98$, and $\theta = 0^\circ$ and $90^\circ$ with $\phi = 0^\circ$. The inset figure is an expanded view of the $M_s = \pm 2$ energy levels in the region of the observed parallel mode EPR transitions of Figure 2 (indicated by arrows).
The EPR signal was performed by varying the temperature parameter in the full diagonalization EPR simulation program. The experimental temperature dependence of the parallel mode EPR signal, along with a theoretical simulation of the temperature-dependent data. The experimental conditions are the same as in Figure 2, except T which is varied for the temperature dependence plot and the power which is varied for the power-dependence plot.

Figure 3. Experimental and theoretical temperature dependence of the EPR signal area vs 1/T for D = 2.10 cm\(^{-1}\) and E = 0.24 cm\(^{-1}\). Inset: power dependence (signal area vs \(1/\sqrt{P}\)) of the EPR signal at 3.2 K; the arrow denotes the nonsaturating power (32 mW) used for the temperature-dependence measurements. Experimental conditions are the same as in Figure 2, except T which is varied for the temperature dependence plot and the power which is varied for the power-dependence plot.

spectrum can be simulated with D and E outside of the range reported here; however, in these cases the temperature dependence does not fit the experimental data.

Upon treatment of MnSOD with azide, a functional inhibitor, the six-line parallel mode CW-EPR signal disappears (data not shown). The Mn\(^{1+}\) ion geometry changes from 5-coordinate in the native sample to a 6-coordinate distorted octahedral geometry with azide forming the sixth ligand at temperatures below 200 K.\(^{12}\) In addition, it is predicted on the basis of MCD studies\(^{6}\) that D will become negative upon azide binding. The lack of an observed parallel mode CW-EPR spectrum in the temperature range of 3–70 K (data not shown) shows that indeed the zero-field splitting parameters are altered, indicating that parallel polarization EPR of the Mn(III) ion provides a sensitive probe of ligation.

Recently, with the observation of an integer spin EPR signal from the oxygen-evolving Mn cluster in photosystem II,\(^{13}\) we have demonstrated that hyperfine-resolved spectra could be obtained on enzymatic Mn clusters. The MnSOD spectrum reported here demonstrates that this holds for monomeric Mn(III) enzymes as well.

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