# EPR SPECTRA OF Fe(III)-SUPEROXIDE DISMUTASE WITH SPECIAL REFERENCE TO THE ELECTRON SPIN RELAXATION TIME OF Fe(III)

Joseph J. VILLAFRANCA

*Pennsylvania State University, Department of Chemistry, University Park, Pennsylvania 16802 USA*

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## 1. Introduction

Superoxide dismutase (SOD) has been isolated from a variety of sources and demonstrates a metal ion requirement for enzymic activity. SOD isolated from bovine erythrocytes and heart [1,2] contains Cu(II) and Zn(II), a dismutase found in *Escherichia coli* [3] and liver mitochondria [4] contains Mn(III) and an additional dismutase isolated from *E. coli* contains Fe(III) [5]. This report consists of electron paramagnetic resonance (e.p.r. studies of the Fe(III)-SOD at 9 and 35 GHz and in particular the environment of the metal ion in the active protein. The results demonstrate a rhombically distorted Fe(III) e.p.r. signal that is unaffected by addition of KCN but which disappears upon addition of reducing agent. Additionally, an upper limit of the electron spin relaxation rate $1/\tau_s$, was evaluated from room temperature e.p.r. measurements on Fe(III)-SOD and compared with the previous n.m.r. estimations of Villafranca et al. [6].

Fe(III)-SOD was isolated from *E. coli* B as outlined by Yost and Fridovich [5] and was found to be identical to a sample generously supplied by Dr F. J. Yost and I. Fridovich. Both samples contained one mole of Fe per 38 000 g of protein and the newly isolated sample had identical solvent proton relaxation rate characteristics as those previously reported [6].

The e.p.r. spectrum of Fe(III)-SOD is given in fig.1 at various temperatures from 6 to 293°K at both 9 and 35 GHz. Previously the e.p.r. spectrum was reported at −100°C [4] in phosphate buffer, pH 7.8. Prominent transitions are noted at ~1500 gauss (9 GHz) and ~6000 gauss (35 GHz) and these arise from the middle Kramers doublet of high spin Fe(III) with a rhombic distortion [7–9]. The splitting is due to deviation from completely rhombic symmetry and the spectrum is fit with $E/D \simeq 0.24$ and $D \geqslant 0.4$.

As the temperature is increased, the intensity of the transitions decrease because of a decrease in the portion of spin in the doublet and an increase in

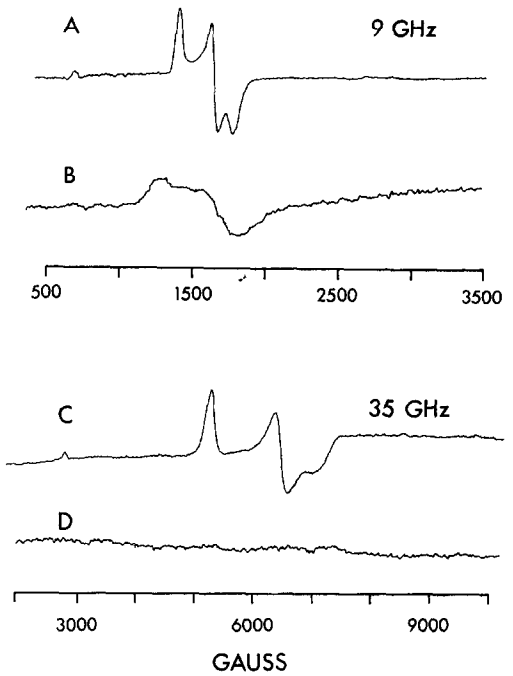


Fig.1. E.p.r. spectra of *E. coli* superoxide dismutase at 9 and 35 GHz. Superoxide dismutase (0.8 mM) was dissolved in 0.1 M Tris-Cl, pH 7.5. Spectra were recorded using a Varian E-12 spectrometer operating at 9.20 or 35.12 GHz. The temperatures for each of the spectra are: (A) 6°K; (B) 293°K; (C) 200°K; (D) 200°K.



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$1/\tau_s$. Few e.p.r. spectra for high spin Fe(III) complexes are reported at room temperature for these reasons but Asakura et al. [10] have shown that useful spectra of Fe(III)-hemoproteins can be obtained in solution.

The spectrum of Fe(III)-SOD was recorded at 1°C (and 25°C) by time averaging the signal and the spectrum is shown in fig.1B. By measuring the line width of bound Fe(III) at 1–25°C (nearly the same range covered in the n.m.r. experiments [6]) a lower limit of $\tau_s$ (upper limit of $1/\tau_s$) was obtained. At 1°C, using a line width of 250 gauss the computed value of $\tau_s$ was $1.3 \times 10^{-10}$ sec. The e.p.r. spectrum at any temperature is unaffected by adding excess KCN. By contrast, the addition of KCN to Cu(II)-SOD alters the Cu(II) e.p.r. spectrum [10,12] and dramatically lowers the longitudinal proton relaxation rates of water [13]. KCN did not alter the proton relaxation rates of Fe(III)-SOD [6]. The e.p.r. spectrum of Fe(III)-SOD disappears upon addition of excess sodium dithionite (fig.1D). This phenomenon was observed when reducing agents were added to the Cu(II)-SOD [14]. Thus, the metal ions of both dismutases can be reduced but the major difference resides in the accessibility of Fe(III) and Cu(II) to solvent exchange and direct interaction with cyanide.

As mentioned previously, an independent measurement of $\tau_s$ was obtained from the peak to peak line width of e.p.r. transitions of Fe(III)-SOD. An upper limit of $1/\tau_s$ can always be computed in this manner but an assumption is made that a single transition is being measured. The value of $\tau_s$ computed in this manner can be used with nmr data to compute the number of rapidly exchanging solvent protons interacting with an enzyme-bound metal ion. However, the $\tau_s$ value computed from e.p.r. data is the transverse electron spin relaxation time ($T_{2e}$) while the Solomon–Bloembergen equations for $1/T_{1p}$ involve both the longitudinal ($T_{1e}$) and transverse ($T_{2e}$) relaxation times [15]. Additionally, $T_{1e}$ can be longer than $T_{2e}$ and both may vary with the strength of the applied magnetic field. From the previous nmr data on Fe(III)-SOD, a correlation time of $3.5 \times 10^{-11}$ sec (table I of ref. [6]) was determined from a frequency dependence of the paramagnetic contribution to the proton longitudinal relaxation rates ($1/T_{1p}$) and this correlation time was evaluated as the diffusion time of water, $\tau_d$, for an outer sphere relaxation mechanism. A numerical analysis of the data also included a contribution from one proton in the inner co-ordination sphere with the correlation time for this process assigned as the electron spin relaxation time, $\tau_s$, of enzyme bound Fe(III). The value quoted for $\tau_s$ was $2.5 \times 10^{-11}$ sec and it was stated that this

Table 1
Electron spin relaxation times of Fe(III) complexes in solution

| Fe(III)-complex | $T_{2e}$ (sec) | $T_{1e}$ (sec) | reference |
|---|---|---|---|
| Transferrin | $\geqslant 3 \times 10^{-10}$ | $\sim 3 \times 10^{-10}$ | [18] |
| Conalbumin | $2 \times 10^{-10}$ | $\sim 2 \times 10^{-10}$ | [19] |
| Superoxide Dismutase | $1.3 \times 10^{-10}$ | | This work |
| EDTA[a] | $3 \times 10^{-9}$ | | [19] |
| $Fe(H_2O)_6^{3+}$ | $6 \times 10^{-11}$ | $1 \times 10^{-10}$ | [15,20,21] |
| Tetra-*p*-tolylporphyrin | | $3 \times 10^{-11}$ | [22] |
| Myoglobin | $6 \times 10^{-11}$ | $3 \times 10^{-11}$[b] | [10,23] |
| Myoglobin-fluoride | $3.4 \times 10^{-10}$ | | [10] |
| Hemoglobin-hydroxide | $6.3 \times 10^{-11}$ | | [10] |
| Cytochrome *c* peroxidase | $< 5 \times 10^{-11}$ | | [10] |
| Catalase | $4 \times 10^{-11}$ | $5 \times 10^{-11}$ | [23] |
| Catalase-cyanide | | $6 \times 10^{-12}$ | [23] |
| $Fe(F)_6^{3+}$ | $6.5 \times 10^{-9}$ | | [21] |
| $Fe(Cl)_4^{1-}$[c] | $\sim 6 \times 10^{-10}$ | | [24] |

[a] Single crystal results.
[b] Myoglobin-formate complex.
[c] Non-aqueous.

was 'typical' of $\tau_s$ values for Fe(III) proteins. $T_{1e}$ can be longer than $T_{2e}$, and therefore the assignment from the Fe(III)-SOD nmr data of $\tau_c = \tau_s = 2.5 \times 10^{-11}$ sec is in error. A correlation of this order of magnitude is most likely due to the diffusion of water molecules into various protein regions near the bound Fe(III) but not directly into the primary co-ordination shell. Thus, proton relaxation appears to be totally due to an outer sphere process with no contribution from inner sphere processes [5]. Two previously documented cases of outer sphere relaxation in metalloproteins are iron–sulfur proteins [16] and Cu–azurin [17].

Values of $T_{2e}$ at ~ 0–30°C have been estimated from e.p.r. line-widths of other high spin Fe(III) containing proteins and smaller molecular complexes. These are listed in table 1. Also listed in table 1 are $T_{1e}$ values obtained from nmr measurements. From the values listed in this table, it seems that the electron spin relaxation times for Fe(III) in a heme environment are generally in the range $10^{-11}$ sec for $T_{1e}$ and $T_{2e}$ (with fluoride complexes of hemoproteins being the only exceptions; see [10]). When Fe(III) is in a non-heme environment the values are $10^{-10}$ sec or longer.

It appears that the proposed mechanism for Fe(III)-SOD involving diffusion of $O_2^-$ into the second co-ordination shell followed by electron transfer remains viable. This hypothesis is supported by the fact that KCN does not diminish the water relaxation rate [6] inhibit the catalytic reaction [25], nor alter the e.p.r. spectrum of bound Fe(III) in SOD. By contrast, Cu(II)-SOD is inhibited by KCN and could involve an inner sphere electron transfer from $O_2^-$ to Cu(II) in this species of SOD.

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