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# An interpretation of EPR spectra of azide ligated superoxide dismutase from Propionibacterium shermanii

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**Abstract.** A self-consistent description of the EPR spectra of the azide ligated SOD is obtained by taking into account the general configuration of the crystal field splitting in the crystal field Hamiltonian. The spread in the rhombicity parameter due to the presence of different conformational substates is introduced.

**Key words:** Superoxide dismutase – EPR – Crystal field symmetry – Spread

EPR spectra of the Fe(III) high spin ion in paramagnetic molecules usually reflect an axial or a rhombic symmetry of the nearest environment. In general, one observes the contributions from the first and the second Kramers doublets resulting from the zero field splitting of the electronic ground state. Often a difficulty arises in the interpretation of the experimental spectra: the position of the maximum of the low-field peak in the region $g \sim 9$ is attributed to the $1 \rightarrow 2$ transition determined by one value of the rhombicity parameter $E/D$, while the sharp peaks at $g \sim 4$ are ascribed to the $3 \rightarrow 4$ transition with another value of $E/D$. In addition, for the simulation of the transition of the middle Kramers doublet it is often necessary to introduce a linewidth nearly one order of magnitude broader than the width used for simulating the lowest Kramers doublet transition (see Gaffney and Silverstone 1993, and reference there). In the case when the zero-field interaction dominates the Zeeman interaction, the combined effect of the spin-orbit and crystal-field mixing of the excited electronic states with the ground state may give rise to contributions of all possible types of the zero field splitting effects. For the $X$-band the requirement is $D \geq 0.4\ \mathrm{cm}^{-1}$.

We shall show that taking due account of the general configuration of the crystal field splitting allows us to describe the EPR spectra of azide ligated SOD from Propionibacterium shermanii without resorting to any artificial assumption concerning the number of the iron species present or different linewidths used in the fitting procedure.

The most general crystal field Hamiltonian customarily used for the $3d^5$ ions with $S = 5/2$ is (Bleaney 1954):

$$\begin{aligned} H = {} & \frac{a}{6}\left[S_\xi^4 + S_\eta^4 + S_\zeta^4 - \frac{1}{5}S(S+1)(3S^2+3S-1)\right] \\ & + D\left[S_z^2 - \frac{1}{3}S(S+1)\right] + E\left(S_x^2 - S_y^2\right) \\ & + \frac{F}{180}\left[35S_z^2 - 30S(S+1)S_z^2 + 25S_z^2\right. \\ & \left. \qquad -6S(S+1) + 3S^2(S+1)^2\right] \end{aligned} \tag{1}$$

where the effective spin $S = 5/2$ coincides with the real spin of the iron ion for the $^6S$ state. Terms in (1) give the following zero field splitting of the six electron states: $a$ describes a crystal field of cubic symmetry, $D$ and $F$ correspond to the axially symmetrical fields of second and forth orders, and $E$ describes the deviation from the axial symmetry in the form of rhombic increment. The axial $z$-axis may not coincide in general with any of the three mutually perpendicular cubic axes $\xi$, $\eta$, $\zeta$. Although a common practice is to neglect the quartic terms in Hamiltonian (1), this is not correct when the magnitude of D is sufficiently small to give rise to the coupling with higher excited states (Oosterhuis 1974). This is typically the case for $D \leq 1.0\ \mathrm{cm}^{-1}$.

The particular form of Hamiltonian (1) is determined by the constraints of symmetry. This may require the coefficients of some zero field splitting operators to become identically zero. In most publications on the $3d^5$ ions the explanation of the EPR spectra is simplified to one of the special cases: either the cubic symmetry prevails (see, e.g. Abragam and Bleaney 1970; Pilbrow 1990), or the strong axial symmetry, with or without rhombic distortion, is predominant. The latter is normally used in the interpretation of the EPR spectra of biological molecules with the high spin ferric ion as an active center (Seidel et al. 1994; Fox et a. 1993; Gaffney et al. 1993). The different values of $E/D$ and of linewidths obtained for the $1 \rightarrow 2$ and $3 \rightarrow 4$ transition are then regarded as different iron species in or-

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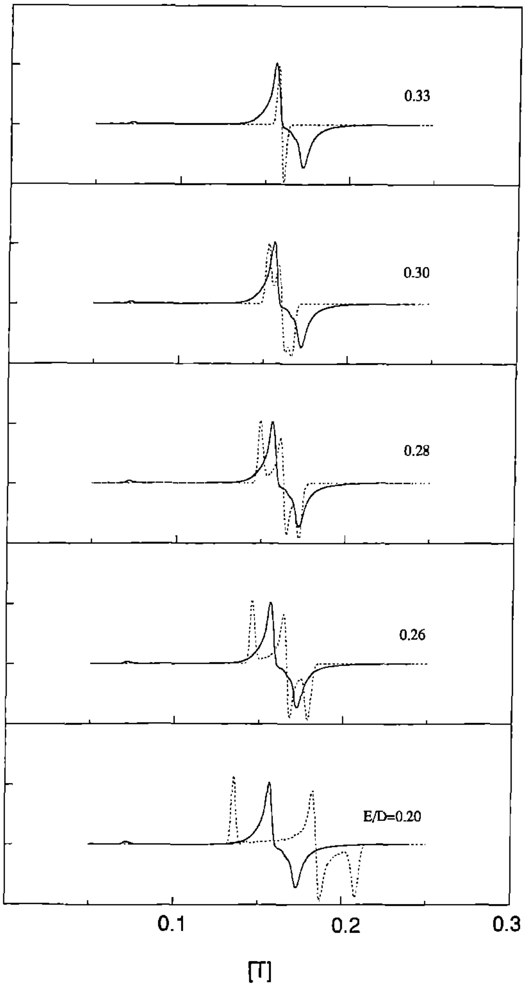

**Fig. 1.** The central part of the EPR spectrum of azide ligated SOD obtained at $T$=4.2 K with $D$=0.745 $cm^{-1}$: the dashed lines show the spectra simulated with the crystal field operators of the second order only in Hamiltonian (1)

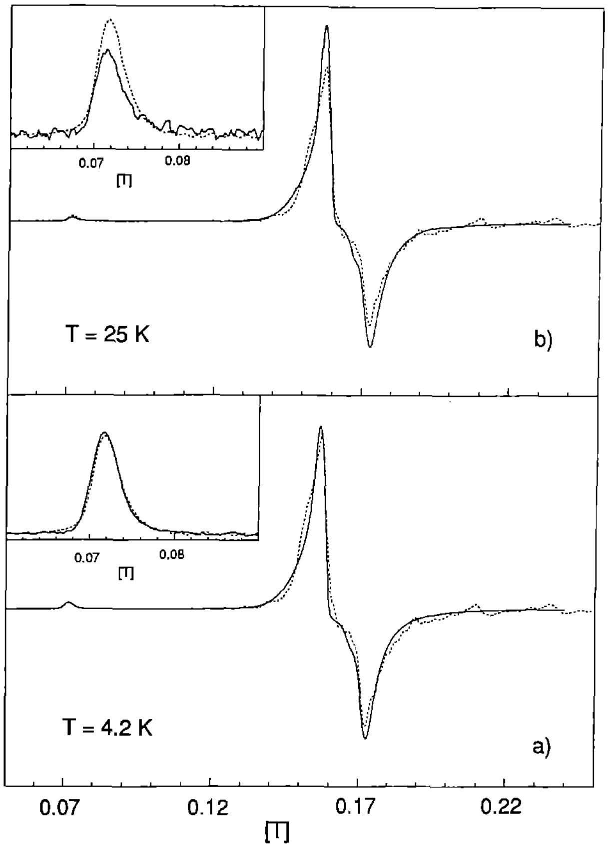

**Fig. 2.** EPR spectra of azide ligated SOD obtained at $T$=4.2 K and $T$=25 K. The dashed lines show the spectra simulated with crystal field Hamiltonian (1): $D$=0.745 $cm^{-1}$, $E/D$=0.286, $a/D$=−0.186, $F/D$=0.266. The line has Lorentzian form and the linewidth is equal to 90 MHz. A Gaussian distribution of the rhombicity parameter $E/D$ with a width equal to 0.044 is used. In this simulation the ($\xi$, $\eta$, $\zeta$) axes coincide with the (x, y, z), axes of the axial field splitting. The insets in the upper left corners show an amplification of the low-field regions of the spectra

der to obtain a reasonable agreement between the calculated and experimental data.

In our simulations presented here we followed the scheme described in detail by Yang and Gaffney (1987). Matrix diagonalization was performed using the program SPHMOSS (Trautwein et al. 1991). In order to determine the resonance magnetic fields $B_{res}$, the secant form of the Newton-Raphson iterative method was used (Gaffney et al. 1993). To simulate a powder spectrum, one should take care to achieve a representative distribution of molecular orientations relative to the fixed directions of the external magnetic field. In our averaging procedure the directions that contribute strongly to the spectra were well sampled, with the averaging step for $\theta$ equal to 1.5°. A Gaussian distribution of $E/D$ value was included to take into account the line broadening and asymmetry in the observed spectra. This distribution reflects the heterogeneity in the geometry of the iron ligands in SOD molecules corresponding to different conformational substates. In order to consider the contribution from this heterogeneity to the lineshape, a series of spectra centered about a particular value of $E/D$ was weighed by a Gaussian intensity factor, and these series were summed numerically. An alternative analysis of the spread of the $E/D$ values may require in principle the consideration of the independent distributions in the energies of the next two excited electronic states (Levin and Brill 1988; Fiamingo et al. 1989). This analysis is in progress.

The EPR spectra of azide ligated SOD from Propionibacterium shermanii were recorded on a Bruker ESP 380 X-band spectrometer (9.8 GHz) equipped with an Oxford ESR 900 helium flow cryostat. The sample temperature was adjusted between 4.2 K and 30 K with an Oxford 3120 temperature controller.

The microwave power was varied between 2 mW and 200 mW, the modulation amplitude was set to 0.5 mT for all spectra. Variable field scans were used to resolve properly the spectral features. The field was monitored with Bruker ER 035 M NMR-Gaussmeter. In some spectra several scans were accumulated to improve the $S/N$-ratio.

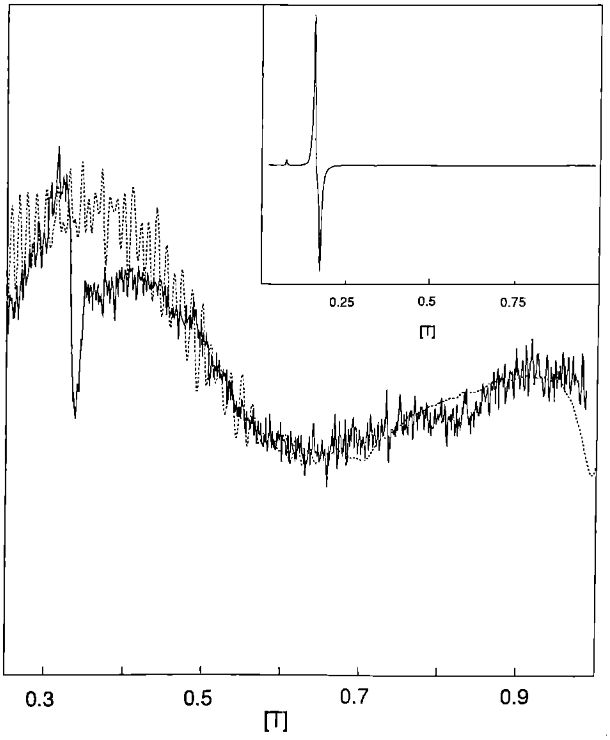

**Fig. 3.** The EPR spectrum of azide ligated SOD at $T$=4.2 K in the high-field region. The dashed curve corresponds to the spectrum simulated with the parameters from Fig. 1. The inset in the upper right corner shows the entire spectrum. The signal at ~0.3 $T$ stems from a Cu-impurity

The simulation of the low-temperature spectrum of azide ligated SOD from Propionibacterium shermanii was started with a set of parameters obtained from the corresponding Mössbauer spectrum. However, the middle Kramers doublet transition could not at all be explained in terms of the crystal field operators of the second order, see Fig. 1. Therefore, we introduced the Hamiltonian (1) in its non-truncated form into our calculations. Figure 2 shows the experimental and simulated EPR spectra of azide ligated SOD obtained at two temperatures, 4.2 K and 25 K. The agreement between the experimental and calculated lineshapes demonstrates the validity of using the complete crystal field Hamiltonian. The same crystal field and linewidth parameters that fit the low-field peaks lead to a good fit in the central part of the spectra. They are common for both temperatures (see legend to Fig. 2). These EPR results prove the presence of a distorted cubic symmetry for each Fe(III) ion site in a SOD molecule. The sign of the cubic field splitting, $a$, corresponds to the relation $a/D<0$ (see, e.g., Oosterhuis 1974; Sweeney et al. 1973). The effect of the Gaussian distribution on the calculated spectra results in a proper fit of the broad tails in the $g\sim4$ region and of the signal asymmetry in the $g\sim9$ region. Last but not least, the broad signals generated by the first Kramers doublet in the high-field region are readily fitted with the same parameter set. This is shown in Fig. 3, where the entire spectrum measured is given.

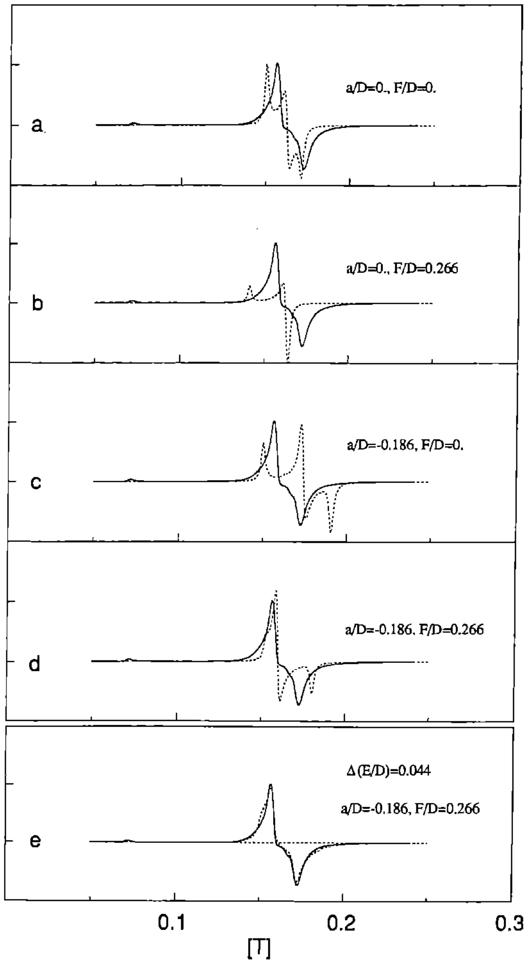

**Fig. 4a–e.** Simulated of the EPR spectrum of azide ligated SOD at $T$=4.2 K. The dashed curve illustrates the effect of the quartic terms in the crystal field Hamiltonian, **a–d**, and of the $E/D$-distribution, **e**. In all the boxes $D$=0.745 cm$^{-1}$, $E/D$=0.286, and the linewidth is equal to 90 MHz

The line position and the lineshape of the calculated EPR spectra depend strongly on the zero field splitting parameters $a$, $D$, $E$ and $F$ and on the width of a Gaussian distribution of $E/D$. The dashed curves in Fig. 4 illustrate the effect of these parameters which are introduced one after another while simulating the second Kramers doublet transition in the low-temperature spectrum. It should be noted that the final agreement between the calculated curve and experiment is only obtained when the distribution of $E/D$ is introduced.

Our analysis of the EPR spectra reveals that the ligands surrounding the Fe(III) ion in the SOD molecule produce an orthorhombic crystal field, with the energy comparable to the strength of the axial and rhombic distortions of the local environment. The importance of a $E/D$ distribution for a good simulation of the spectra demonstrates once more the existence of conformational substates in proteins.

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