

Definitive Assignment of the *g* Tensor of [Fe(OEP)(NO)] by Single-Crystal EPR

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Single-crystal EPR measurements have been performed on the triclinic form of [Fe(OEP)(NO)] (Ellison, M. K.; Scheidt, W. R. *J. Am. Chem. Soc.* **1997**, *119*, 7404) and on the isomorphous cobalt derivative [Co(OEP)(NO)] (Ellison, M. K.; Scheidt, W. R. *Inorg. Chem.* **1998**, *37*, 382) which has been doped with [Fe(OEP)(NO)]. Principal values of the *g* tensor determined at room temperature are  $g_{\max} = 2.106$ ,  $g_{\text{mid}} = 2.057$ , and  $g_{\min} = 2.015$ . The principal direction associated with the minimum *g* value lies  $8^\circ$  from the Fe–N(NO) direction,  $2^\circ$  from the normal to the heme plane, and  $42^\circ$  from the N–O direction. The direction associated with the maximum *g* value lies  $9^\circ$  from the normal to the Fe–N–O plane. The fact that the direction of  $g_{\min}$  is near the Fe–N(NO) direction is consistent with the dominant role of spin–orbit coupling at the iron atom in determining the *g* tensor and with the picture of the electronic structure of the compound from restricted calculations, which makes the half-filled orbital mostly  $d_z^2$  on the iron atom. The hyperfine tensor is nearly isotropic and was only resolved in the doped samples. Principal values of the *A* tensor determined at room temperature are 40.9, 49.7, and 42.7 MHz. Principal values of the *g* tensor determined from the doped samples at 77 K are  $g_{\max} = 2.110$ ,  $g_{\text{mid}} = 2.040$ , and  $g_{\min} = 2.012$ . Principal values of the *A* tensor are 42.5, 52.8, and 44.1 MHz at 77 K. The small change in *g* values with temperature is in contrast to the large temperature dependence on *g* values observed in samples of MbNO (Hori et al. *J. Biol. Chem.* **1981**, *256*, 7849).

## Introduction

The study of (nitrosyl)iron(II) hemes by EPR spectroscopy goes back nearly 35 years. The early interest in these species stemmed from their utility as paramagnetic models for the biologically important oxygenated heme proteins that are EPR inactive. Single-crystal EPR studies of both HbNO<sup>1,2</sup> and MbNO<sup>3</sup> are available, as well as studies of (nitrosyl)iron(II) porphyrins and hemoproteins in fluid solutions and in frozen solutions.<sup>4–13</sup> More recently, NO has been discovered to be a key molecule in important biological functions such as neurotransmission, blood pressure regulation, inhibition of platelet aggregation, and cytotoxic action of macrophages.<sup>14</sup> Most of these physiological processes involve the interaction of NO with heme proteins and are amenable to EPR studies. However, no

studies have been done which orient the magnetic parameters of the system in an [Fe(Porph)(NO)] coordinate system without making unverifiable assumptions.

We have recently communicated the crystal structures of two crystalline forms of [Fe(OEP)(NO)].<sup>15,16</sup> One of these, a triclinic form, forms large single crystals and has two molecules/unit cell related by an inversion center. Thus, it has only one magnetically distinct site in the crystal lattice. This material affords an excellent opportunity to fix the magnetic parameters of a (nitrosyl)iron(II) porphyrin in the molecular coordinate system. Moreover, since an isomorphous, EPR inactive, cobalt nitrosyl has also been prepared and characterized,<sup>17</sup> a magnetically dilute single-crystal sample can be prepared by doping the [Co(OEP)(NO)] sample with a small percentage of [Fe(OEP)(NO)].

## Experimental Section

**Sample Preparation.** Single crystals of the triclinic form of [Fe(OEP)(NO)] were obtained as described previously.<sup>15,16</sup> Single crystals of [Co(OEP)(NO)] were obtained as described previously<sup>17</sup> except the crystals now contain either ~5% or ~0.6% [Fe(OEP)(NO)]. The orientation of single crystals of [Fe(OEP)(NO)] and [Co(OEP)(NO)] crystals doped with [Fe(OEP)(NO)] were determined by indexing the faces of the crystals on a Nonius FAST area-detector diffractometer and then mounting them on small squares of fused silica so that the orientation of the crystal with respect to the silica squares was known. The fused silica squares contained E' centers used to calibrate the magnetic field.<sup>18</sup> The Fe-doped crystals of [Co(OEP)(NO)] were also mounted on smaller plastic (PE) cubes with an unknown orientation.

**EPR Spectroscopy.** All EPR spectra were measured with a conventional Varian E-line spectrometer, which operates near 9.0 GHz.

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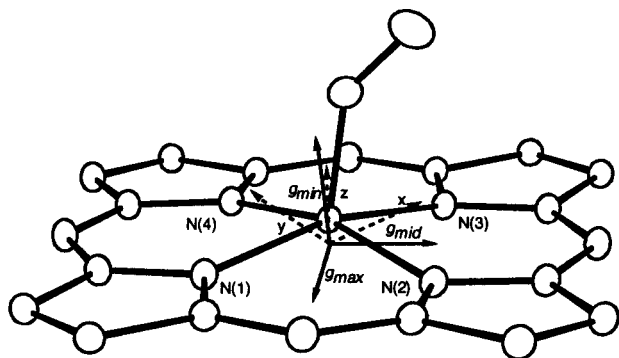
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**Figure 1.** ORTEP diagram of [Fe(OEP)(NO)] depicting the orientations of the principal axes of the  $\mathbf{g}$  tensor with respect to the molecular coordinate system. The ethyl groups and hydrogen atoms are omitted for clarity.

A Varian Multipurpose Cavity was used as the resonant element. EPR spectra were obtained at room temperature for crystals of [Fe(OEP)(NO)] and the doped crystals of [Co(OEP)(NO)]. Orientation-dependent spectra at room temperature were obtained by establishing a laboratory coordinate system on the silica square and rotating the square (thus, the crystal) about three orthogonal axes. A single-circle goniometer was used which had a Teflon rod to support the sample. Orientation-dependent spectra were similarly obtained at 77 K on doped crystals of [Co(OEP)(NO)] mounted on plastic cubes. A coordinate system was also established on the plastic cube which carried the crystal. The cube was glued to the end of a piece of glass tubing that extended into a fused silica finger Dewar flask filled with liquid nitrogen. The sample was rotated with a single-circle goniometer. For all samples, spectra were measured at least every 30° and, for most rotations, every 15°, over a 180° range.

Electronic structure calculations on [Fe(OEP)(NO)] were performed, using the *Gaussian 94* suite of programs,<sup>19</sup> in order to compare the calculated spin density distribution parameters with the observed hyperfine parameters and also to compare the spin density predictions with predictions from an earlier calculation by a different technique.<sup>20</sup> The calculations performed were restricted DF calculations (B3LYP) at the observed geometry, using the LanL2DZ effective core potential basis set.

## Results

EPR spectra were obtained on single-crystal samples of the triclinic form of [Fe(OEP)(NO)] where there is only a single magnetically distinct site. The positions of the resonance absorptions were recorded as the crystal was rotated over 180° in three orthogonal directions. The  $\mathbf{g}$  tensor was obtained in the laboratory coordinate system by the method first discussed by Schönland.<sup>21</sup> Since the orientation of the crystal in the laboratory system was known, the  $\mathbf{g}$  tensor could be transformed to a coordinate system fixed in the molecule (see Figure 1). The molecular coordinate system is defined as follows:  $z$  is perpendicular to the mean porphyrin nitrogen-atom plane, pointing toward the nitrosyl group,  $x$  is in that plane, pointing toward N(3), and  $y$  is oriented to make an orthogonal right-handed system. Table 1 displays the principal  $g$  values obtained

**Table 1.** Principal  $g$  Values and Principal Axes of [Fe(OEP)(NO)]

$g$ value	$x$	$y$	$z$
2.106	0.765	0.623	-0.163
2.057	0.644	-0.745	0.174
2.015	0.013	0.238	0.971

from a crystal of pure [Fe(OEP)(NO)] as well as the direction cosines of the principal axes in the coordinate system defined above. The principal direction associated with the minimum  $g$  value lies 8° from the Fe–N(NO) direction, 2° from the normal to the heme plane defined by the nitrogen atoms, and 42° from the N–O bond direction. The direction associated with the maximum  $g$  value lies 9° from the normal to the Fe–N–O plane. We estimate the uncertainty in the principal  $g$  values to be  $\pm 0.001$  and the uncertainty in angles to be  $\pm 5.0^\circ$ . Hyperfine structure was not resolved in the spectra of pure [Fe(OEP)(NO)].

The isomorphous cobalt derivative which is EPR inactive provides an excellent opportunity to study the angular dependence of a magnetically dilute sample of [Fe(OEP)(NO)] by doping the cobalt sample with a small amount of the iron derivative. EPR spectra were obtained on single crystals of [Co(OEP)(NO)] doped with 0.6% [Fe(OEP)(NO)] at room temperature and at 77 K where the spectral resolution is improved and hyperfine splittings could be resolved. The orientation of the crystals that were used for the 77 K spectra in the laboratory system was not known. The data were used to find the principal values of the  $\mathbf{g}$  tensor in the laboratory coordinate system and the principal values and orientation of the  $\mathbf{A}$  tensor in the principal axis system of the  $\mathbf{g}$  tensor. The doped crystals of [Co(OEP)(NO)] had somewhat poorly resolved hyperfine structure at room temperature and much better resolved hyperfine structure at 77 K. The hyperfine tensor was measured and analyzed at both temperatures. Analysis of the data from the doped crystals at room temperature yields principal  $g$  values of 2.103, 2.062, and 2.012. We have not referred these to a molecular coordinate system with a crystallographically oriented single crystal.

The principal values of the hyperfine tensor at room temperature, from the doped crystals, are 40.9, 49.7, and 42.7 MHz. The principal axes are not collinear with the principal axes of the  $\mathbf{g}$  tensor but are calculated to lie about 30° from them. These angles are not especially accurately determined since, as can be seen, the hyperfine tensor is nearly isotropic. The principal axes of the first principal value listed is closest to the direction of the maximum  $g$  value, the second to the intermediate  $g$  value, and so on.  $\bar{A} = 44.44$  MHz. EPR spectra obtained at 77 K have greater angular uncertainties than those taken at room temperature. However, reproducibility of results from replicate experiments is acceptable and the larger value of  $A$  is obvious in the spectra. We obtain principal  $g$  values of 2.110, 2.040, and 2.012 and principal  $A$  values of 42.5, 52.8, and 44.1 MHz, with  $\bar{A} = 46.5$  MHz. The association of principal  $A$  values with principal  $g$  values is the same, but the principal axes now more nearly coincide. They are within 10° of each other.

## Discussion

The most obvious finding in this work is the orientation of the principal axes of the  $\mathbf{g}$  tensor, which has not been available from previous work. The fact that  $g_{\min}$  is close to the free electron value is consistent with the dominant role of spin-orbit coupling at the iron atom in determining the  $\mathbf{g}$  tensor and with the picture of the electronic structure of the compound from restricted calculations, which makes the half-filled orbital

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mostly  $d_{z^2}$  on the iron atom. The fact that  $A(^{14}\text{N})$  is nearly isotropic is also consistent with the picture of the electronic structure from the  $g$  tensor, since the half-filled orbital has a amplitude on the nitrosyl nitrogen atom.

[Fe(OEP)(NO)] is a nitrosyl compound of type  $\{\text{MNO}\}^7$  in the system of representation of the electronic structure that was introduced by Enemark and Feltham.<sup>22</sup> The general electron counting formalism is represented by  $\{\text{MNO}\}^n$  where the total number of electrons ( $n$  in the notation) is the number of  $d$ -electrons from the metal, always evaluated by considering NO as neutral, plus the one unpaired electron from the  $\pi^*$  orbitals of neutral NO. Species denoted as  $\{\text{FeNO}\}^7$  are regarded as being derived from iron(II) centers and NO. Both magnetic susceptibility measurements and EPR measurements show the compound to be low spin with one unpaired electron.

Hoffmann and co-workers have provided a qualitative molecular orbital description of compounds that have a strong interaction between a transition metal atom and a diatomic ligand (AB).<sup>23</sup> In their model, the transition metal atom is also bonded to four other identical ligands that do not interact strongly with the metal atom, in a pyramidal geometry. They use a coordinate system with the  $z$  axis normal to the plane of the four identical ligands and the  $x$  and  $y$  axis in the planes that contain the  $z$  axis and pairs of the ligands. They conclude that the unpaired electron in a low-spin  $\{\text{MAB}\}^7$  compound occupies an antibonding molecular orbital that is composed of a  $d_{z^2}$  orbital on the transition metal atom and an orbital on the diatomic ligand that is a combination of a nonbonding  $\sigma$  orbital (essentially,  $sp$  hybrids on the two atoms of the diatomic, pointing away from the bond) and the diatomic  $\pi$ -bonding orbital that lies in the plane of the transition metal atom and the two atoms of the diatomic. As the MAB angle goes from  $180^\circ$  toward  $90^\circ$ , the amount of  $\pi$  orbital in the combination increases. At  $180^\circ$ , the ligand orbital is entirely the nonbonding  $\sigma$  orbital.

Since [Fe(OEP)(NO)] is best described as an  $\{\text{FeNO}\}^7$  species, in a simple crystal field model, it must be considered a  $d^7$  compound. In a crystal field model of low-spin  $d^7$  compounds that have the  $d_{z^2}$  orbital half-filled, the  $g$  factors are determined primarily by interaction between the ground state and states in which an electron is promoted from the filled  $d_{xz}$  or  $d_{yz}$  orbital into the  $d_{z^2}$  orbital.

Expressions for the  $g$  factors of low-spin  $d^7$  compounds have been published by several workers. We have used the work of McGarvey.<sup>24</sup> If McGarvey's expressions for the  $g$  factors of a low-spin  $d^7$  compound that has the  $d_{z^2}$  orbital half-filled are simplified by retaining only terms that are linear in the coupling coefficients between the ground state and excited states, they become

$$g_{z'z'} = g_0 \quad (1)$$

$$g_{x'x'} = g_0 + 6\xi/\Delta_1 \quad (2)$$

$$g_{y'y'} = g_0 + 6\xi/\Delta_2 \quad (3)$$

In these equations,  $g_0$  is the free-electron  $g$  factor, 2.0023,  $\xi$  is the metal 3d one-electron spin-orbit coupling constant,  $\Delta_1$  is the difference in energy between the ground state and the excited state in which the unpaired electron is found in the  $d_{y'z'}$  orbital, and  $\Delta_2$  is the difference in energy between the ground state and the excited state in which the unpaired electron is found

in the  $d_{x'z'}$  orbital.<sup>25</sup> In the coordinate system for these equations,  $z'$  is the  $z$  coordinate of the molecular coordinate system (normal to the mean plane of the porphyrin nitrogen atoms),  $y'$  lies in that mean plane and normal to the Fe-N-O plane, and  $x'$  makes an orthogonal coordinate system.

Although eqs 1–3 were obtained from a crystal-field model of a low-spin  $d^7$  compound, equations of the same form will apply under the following conditions: (1) The half-filled orbital has considerable  $d_{z^2}$  atomic orbital amplitude on the metal, plus amplitude on ligand atomic orbitals. (2) The filled orbitals include orbitals with considerable  $d_{xz}$  and  $d_{yz}$  amplitudes. (3) Unfilled orbitals with considerable  $d_{xz}$  or  $d_{yz}$  amplitude may be ignored. The first two conditions arise because the iron 3d spin-orbit coupling constant is much larger than the spin-orbit coupling constants of any of the ligand orbitals. The 3d coupling constant of  $\text{Fe}^+$  ( $d^7$ ) is  $335 \text{ cm}^{-1}$ , for instance, and the oxygen 2p coupling constant is  $70 \text{ cm}^{-1}$ .<sup>26</sup> Nitrogen and carbon have still smaller 2p coupling constants. The third condition arises because a crystal-field model does not allow states in which the unpaired electron is promoted to unfilled  $d_{xz}$  or  $d_{yz}$  orbitals.

When our data are compared with the predictions of eqs 1–3, it is seen that, in [Fe(OEP)(NO)], the orbital that contains the unpaired electron has considerable amplitude on the  $d_{z^2}$  of the iron atom. This conclusion comes from the fact that  $g_{zz}$  is close to  $g_0$  and that the two in-plane principal  $g$  values are larger than  $g_0$ . Thus, our data are consistent, qualitatively, with either the traditional crystal-field picture of a low-spin  $d^7$  compound or with the conclusions of Hoffmann and co-workers.

We can obtain a somewhat more complete comparison of our results with the conclusions of Hoffmann and co-workers by comparing the ordering of the in-plane  $g$  values with the ordering that is expected from the work of the Hoffmann group. According to the analysis of Hoffmann and co-workers, the orbitals that are described as  $d_{x'z'}$  and  $d_{y'z'}$  in the crystal-field model behave as follows: When the M-A-B angle is  $180^\circ$ , these orbitals have the same energy and are bonding combinations of the metal  $d_{x'z'}$  and  $d_{y'z'}$  orbitals and the AB  $\pi^*$  orbitals. As the M-A-B angle decreases, the orbital based on  $d_{y'z'}$  stays at about the same energy but the orbital based on  $d_{x'z'}$  rises in energy. Eventually, when the M-A-B angle becomes  $90^\circ$ , these orbitals become bonding and antibonding combinations of  $d_{x'y'}$  with one of the AB  $\pi^*$  orbitals.

For our purposes, the main consequence of the behavior of the  $d_{x'z'}/d_{y'z'}$ -like orbitals is that, in [Fe(OEP)(NO)],  $\Delta_1 > \Delta_2$ . Thus, one expects that  $g_{y'y'} > g_{x'x'}$ . This is confirmed by our observations.  $g_{\text{max}}$ , the largest principal  $g$  value, is associated with a principal direction that is very nearly collinear with  $y'$ .

The unpaired electron is in an orbital that may have amplitude on the 2s orbital of the nitrosyl nitrogen atom. This, presumably, accounts for the isotropic  $^{14}\text{N}$  hyperfine coupling constant, which has a value of 44.4 MHz at room temperature. An unpaired electron in the 2s orbital of a nitrogen atom is calculated to produce an isotropic hyperfine coupling constant of 1811 MHz,<sup>27</sup> so the 2s spin density on the nitrosyl nitrogen atom is 0.025. The purely anisotropic part of the  $^{14}\text{N}$  hyperfine tensor is roughly axial about the principal axis that is closest to  $y'$ . To obtain an estimate of the 2p spin density without making a model of the spin density distribution about the nitrosyl nitrogen atom, we have assumed that the  $^{14}\text{N}$  hyperfine tensor is exactly axial. To

(25)  $\Delta_1 = (d_{x'y'})^2(d_{x'z'})^2(d_{y'z'})^1(d_{z^2})^2$ ;  $\Delta_2 = (d_{x'y'})^2(d_{x'z'})^1(d_{y'z'})^2(d_{z^2})^2$ .

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do this, we have averaged the two negative principal values and obtained a purely anisotropic tensor ( $-2.65$  MHz,  $5.30$  MHz,  $-2.65$  MHz). An unpaired electron in a  $2p$  orbital of  $^{14}\text{N}$  produces a purely anisotropic tensor ( $-55.52$  MHz,  $111.04$  MHz,  $-55.52$  MHz),<sup>26</sup> so the nitrogen  $2p$  spin density is  $0.048$ . The total spin density on the nitrosyl nitrogen atom is  $0.073$ . The nitrosyl oxygen atom spin density is probably about half of this, as a rough estimate, so the total nitrosyl spin density may be roughly estimated to be  $0.11$ . This leaves a spin density of  $0.89$  on the iron atom and the porphyrin. Of course this partitioning of spin density assumes that the spin densities at the various points in the molecule come from a single orbital that contains the unpaired electron.

We have attempted to go beyond qualitative models of the electronic structure of  $[\text{Fe}(\text{OEP})(\text{NO})]$  by performing a restricted density functional (DF) calculation (rob3lyp) on  $\text{Fe}(\text{P})(\text{NO})$  with the LanL2DZ basis set. We used the *Gaussian 94* suite of programs.<sup>19</sup> The LanL2DZ basis set is an ECP basis set and has no core orbitals on the heavy atoms. The spin density of the molecule is calculated to be localized almost entirely on the  $\text{FeNO}$  group, with a spin density of  $0.800$  on the iron atom, a spin density of  $0.096$  on the nitrogen atom, and a spin density of  $0.043$  on the oxygen atom. The  $^{14}\text{N}$  isotropic coupling constant is calculated to be  $42.2$  MHz. This agreement with the experimental value is better than can be expected from the quality of the basis set; however, it does support the result from the calculation that nearly all of the unpaired spin density is on the iron atom.

The orbitals from the DF calculation are molecular orbitals, of course, and have amplitudes on many atoms of the molecule. The orbital of the unpaired electron has large amplitudes only on the atoms of the  $\text{FeNO}$  group, however. The primary qualitative difference between this orbital and the orbital deduced by Hoffmann and co-workers is that the orbital from the DF calculation has significant  $d_{x'z'}$  amplitude in addition to its  $d_{z^2}$  amplitude. Also, the filled orbitals that are  $d_{x'z'}/d_{y'z'}$  in the crystal field model become mixtures of  $d_{z^2}$ ,  $d_{x'z'}$ , and  $d_{y'z'}$ . Finally, there are virtual orbitals with amplitudes on  $d_{z^2}$ ,  $d_{x'z'}$ , and  $d_{y'z'}$ .

To assess the effect of the differences between the DF description of the molecule and the simpler models we have

used, we created a crystal field model. In the model, the three orbitals of significance for the crystal field calculation were taken to be composed entirely of iron atom  $3d$  orbitals, but each orbital had the ratio of contributions that was obtained from the DF calculation. These orbitals were then used to calculate the principal  $g$  factors at the level of approximation of eqs 1–3. Qualitatively, there is very little difference from eqs 1–3. The minimum  $g$  value still lies nearly along  $z'$  and is close to  $g_0$ . The other two  $g$  values are larger than  $g_0$ . So, to the extent that one can ignore the contribution of the virtual orbitals to the  $g$  factors, the more complete description does not change the interpretation of the observed magnetic parameters.

The principal direction that is associated with the minimum  $g$  value lies along  $d_{z^2}$  at least in a crystal field model of the electronic structure of the iron atom. In  $[\text{Fe}(\text{OEP})(\text{NO})]$ , this principal direction lies only  $2 \pm 5^\circ$  from the normal to the mean porphyrin plane.

The dependence of the magnetic parameters on temperature, which we have observed to be of modest size, is probably due to the  $\text{Fe}-\text{N}-\text{O}$  bending vibration or frustrated pseudorotation of the  $\text{NO}$  group around the  $\text{Fe}-\text{N}$  bond, but we have no evidence to support this proposal. This small change in  $g$  values with temperature is in contrast to the large temperature dependence on  $g$  values observed in samples of  $\text{MbNO}$  and other hemoproteins.<sup>3,7</sup>

**Summary.** Single-crystal EPR studies of  $[\text{Fe}(\text{OEP})(\text{NO})]$  show unambiguously that the unpaired electron in the compound is associated mostly with the iron atom. It occupies an orbital that is mostly  $d_{z^2}$  on the iron atom. This assertion is supported both by the  $\mathbf{g}$  tensor and by nitrosyl  $^{14}\text{N}$  hyperfine tensor of the compound. The principal axis that corresponds to the minimum  $g$  value is the  $z$  axis of a coordinate system for the metal atom orbitals. This axis is very nearly along the normal to the mean porphyrin plane.

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