

ENDOR Studies of Nitrosyl Complexes of Iron Porphyrins

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Because of their paramagnetic properties nitrosyl complexes of hemoproteins have been widely investigated by EPR spectroscopy, to obtain insight into the electronic and stereochemical nature of oxygen binding in oxyhemoproteins which are not accessible to EPR. For obtaining their fundamental aspects, investigations using model complexes seem valuable because various choices of the fifth ligand are possible. In this paper we report effects of the fifth ligand on N(NO) hf interactions in nitrosyl complexes of octaethylporphinato- and tetraphenylporphinatoiron(II), FeOEP(B)(NO) and FeTPP(B)(NO) (B = nitrogen base), investigated by ENDOR spectroscopy. The ENDOR spectroscopy is a useful technique for obtaining hf coupling data because it sometimes resolves hf couplings hidden within line widths in EPR spectra.

Experimental

Sample solutions for ENDOR measurements were prepared by dissolving FeOPE(NO) and FeTPP(NO) isolated according to Scheidt *et al.* [1, 2] in toluene containing a nitrogen base in large excess to the nitrosyl complexes on a vacuum line. EPR spectra were recorded at 50–60 K on a Varian E112 X-band EPR spectrometer, and ENDOR measurements were performed at 15–25 K by combining the EPR spectrometer with a Varian E1700 ENDOR device. The temperatures were controlled by an Oxford ESR 9 cryostat.

Results and Discussion

ENDOR signals of the nitrosyl nitrogen were observed in the frequency range 16–33 MHz. Examples of the spectra are shown in Fig. 1, obtained by setting the magnetic field at the positions

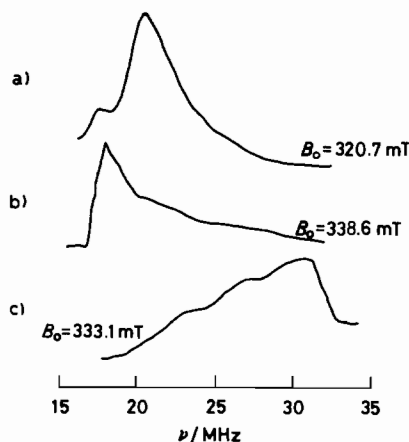


Fig. 1. $^{14}\text{N}(\text{NO})$ ENDOR spectra of FeOEP(4NH₂Py)(NO) in toluene at 20 K. The spectra were recorded by setting the magnetic field at the positions corresponding to a) g_x ($B_0 = 320.7$ mT), b) g_y ($B_0 = 338.6$ mT) and c) g_z ($B_0 = 333.1$ mT) in the EPR spectrum.

corresponding to g_x , g_y and g_z values in the EPR spectra, respectively. The peak position of each ENDOR signal changes slightly by shifting the magnetic field around those field set positions. The A_x , and A_y , values listed in Table I were determined from the peak positions showing the lowest rf frequency in the measurements of the field sets at the g_x and g_y regions. The A_z values were determined from those showing the highest rf frequency in the measurements of the field sets at the g_z region. In the case of FeTPP(B)(NO), as the ENDOR signals obtained from the field set at the g_z region were too broad and weak to record them with good S/N ratio, the coupling constants were determined from the hf splittings at the g_z region in the EPR spectra observed for the ^{15}N enriched nitrosyl complex. The hf coupling data obtained from the EPR spectra were converted to the values for ^{14}N (Table I). The most A_z , values obtained from the ENDOR spectra for FeOPE(B)(NO) showed slight differences from the values obtained from the hf splittings in the EPR spectra. Such differences may be attributed to discord between the g and hf tensor axes.

Obtaining the three components of the nitrogen hf couplings by ENDOR spectroscopy, it has become possible to divide the data to the isotropic and anisotropic components. Anisotropic coupling tensors so obtained are not axial symmetric and are further divided into two axial symmetric tensors with the principal axes of z' and x' , respectively. In view of the results of the EPR analysis for the nitrosyl-myoglobin single crystal [3], the former may be

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TABLE I. $^{14}\text{N}(\text{NO})$ Hf Coupling Constants for FeTPP(B)(NO) and FeOEP(B)(NO) in Toluene.^a

| | Ax. Lig. (B) ^b | A_x , | A_y , | A_z , | A_i | $A_{\text{ani}}(p\sigma)$ | $A_{\text{ani}}(p\pi)$ |
|-------|---------------------------|---------|---------|---------|-------|---------------------------|------------------------|
| FeTPP | N-MeIm | 37.37 | 33.14 | 63.3 | 44.6 | 20.0 | 2.8 |
| | 4NH ₂ Py | 37.99 | 35.77 | 62.8 | 45.5 | 18.0 | 1.5 |
| | Py | 38.03 | 35.79 | 62.1 | 45.3 | 17.6 | 1.5 |
| | 4COCH ₃ Py | 39.52 | 37.77 | 59.9 | 45.7 | 14.8 | 1.2 |
| | 3CNPy | 40.66 | 38.91 | 59.8 | 46.5 | 12.8 | 1.2 |
| | 4CNPy | 40.08 | 38.98 | 60.6 | 46.5 | 14.4 | 0.7 |
| FeOEP | 4NH ₂ Py | 39.45 | 34.58 | 61.23 | 45.09 | 17.77 | 3.25 |
| | Py | 39.51 | 35.68 | 60.45 | 45.21 | 16.52 | 2.55 |
| | 4COCH ₃ Py | 39.76 | 36.37 | 59.52 | 45.22 | 15.43 | 2.26 |
| | 4CNPy | 40.24 | 38.46 | 59.23 | 45.98 | 13.84 | 1.18 |

^aIn units of MHz. ^bN-MeIm: N-methylimidazole, Py: pyridine.

TABLE II. Electron Spin Distribution on Nitrosyl Nitrogen in FeTPP(B)(NO) and Nitrosylmyoglobin.

| Ax. Lig. (B) | ρ_s | $\rho_{p\sigma}$ | $\rho_s/\rho_{p\sigma}$ | |
|--------------------------|----------|------------------|-------------------------|-----------------------|
| N-MeIm | 0.029 | 0.21 | 0.14 | |
| 4NH ₂ Py | 0.030 | 0.19 | 0.16 | |
| Py | 0.029 | 0.18 | 0.16 | |
| 4COCH ₃ Py | 0.030 | 0.15 | 0.19 | |
| 3CNPy | 0.030 | 0.13 | 0.23 | |
| 4CNPy | 0.030 | 0.15 | 0.20 | |
| MbNO Cryst. ^a | | | | $\angle(\text{FeNO})$ |
| 293K | 0.03 | 0.23 | 0.13 | 153° |
| 77K | 0.03 | 0.13 | 0.23 | 109° |

^aReference 3.

considered as arising from the spin density on the nitrogen p orbital directed to the Fe–NO bond and the latter the spin density on the nitrogen p orbital parallel to the porphyrin plane. In Table I, these two anisotropic components are designated as $A_{\text{ani}}(p\sigma)$ and $A_{\text{ani}}(p\pi)$. On the other hand, the isotropic hf coupling constants can be attributed to the interaction with the spin density on the nitrogen $2s$ orbital. Table II lists the spin densities evaluated from these hf components using the parameters $A_s = 1540$ MHz and $A_p = 47.8$ MHz given for a pure s orbital and for a pure p orbital on a nitrogen atom, respectively [4].

Recently Kobayashi *et al.* [5] reported that the A_z values obtained from EPR spectra for the nitrosyl complex of protohemin increase with increase of basicity of the fifth ligand base. In the present case, increase of the A_z values was also observed. Interestingly, Tables I and II show that such an increase in

the A_z values arises from increase in the spin density on the nitrogen p orbital, and contrarily the spin density on the nitrogen $2s$ orbital slightly decreases. Such changes of the s/p ratio in the spin distribution by the fifth ligand indicate that the fifth ligand affects not only the total spin density on the nitrosyl nitrogen but also the nature of the interaction between the iron and the nitrosyl ligand.

Hori *et al.* [3] showed in the single crystal EPR studies of nitrosylmyoglobin that the Fe–N–O angle changes with temperature, accompanying changes of the spin density distribution on the nitrogen orbitals in the nitrosyl ligand. As Table II shows, the spin density distribution on the nitrogen orbitals in the present system corresponds well to that in the myoglobin system. That is, the spin distribution in FeTPP(N–MeIm)(NO) corresponds to that in nitrosylmyoglobin at 293 K, while that in FeTPP(3CNPy)(NO) to that in the myoglobin system at 77 K. These results strongly suggest that Fe–NO conformation in the present system may vary with the axial bases. In other words, the results imply that the changes of the Fe–NO conformation with temperature in the myoglobin system may relate to changes in the strength of interaction between the iron and the fifth ligand His(F8).

In the myoglobin system, appreciable changes of the g tensor were observed by the change of Fe–NO conformation with temperature. In the present case, however, such large changes in the g tensors were not observed by the change of the axial base; for example the g values for FeTPP(N–MeIm)(NO) are $g_x = 2.075$, $g_y = 1.970$ and $g_z = 2.003$ and those for FeTPP(4–CNPy)(NO) are $g_x = 2.085$, $g_y > 1.98$ and $g_z = 2.005$. Further studies are required to obtain clearer understanding of the correlation between the Fe–NO conformation and the nature of Fe-axial base interaction.

It should be noted that the change from the OEP to the TPP ligand does not greatly affect the Fe-NO interaction.

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