

## NO Orientation and Tilting in (Nitrosyl)iron(II) Deuteroporphyrin IX

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To investigate issues concerning the coordination of the nitrosyl ligand in naturally occurring hemes, we report the spectroscopy and X-ray structure of five-coordinate [Fe(Deut)(NO)]. Bonding parameters are comparable with those observed for previously characterized synthetic porphyrin complexes of this type. The asymmetric pattern of the peripheral substitution of the porphyrin core allows us to examine aspects associated with ligand binding and orientation previously unobserved in the symmetrical synthetic porphyrins. The nitrosyl is found to be oriented in the direction of the less basic pyrrole rings. This observed orientation of the NO is considered in reference to those orientations reported in a series of related protein structures. Off-axis tilting, a property associated with ordered (nitrosyl)iron(II) porphyrinates, is also investigated.

A number of critical physiological processes are dependent on the coordination of nitric oxide (NO) to heme.<sup>1</sup> Although structures for a number of iron porphyrinate nitrosyls have been reported,<sup>2</sup> all use highly symmetric porphyrin derivatives. We report the structure of [Fe(Deut)(NO)],<sup>3</sup> the first structurally characterized NO derivative using a naturally occurring porphyrin.<sup>4</sup> [Fe(Deut)(NO)], an {FeNO}<sup>7</sup> complex,<sup>5</sup> allows us to further explore stereochemical issues previously observed with synthetic porphyrin derivatives.

Recent high-precision structures<sup>6</sup> of {FeNO}<sup>7</sup> porphyrinate complexes have shown that the Fe–N(NO) bond is tilted off the normal to the heme plane, a property apparently arising purely from ligand bonding interactions. This off-axis tilting has been observed for all ordered porphyrins (all

synthetic) regardless of coordination number.<sup>6,7</sup> The five-coordinate {FeNO}<sup>7</sup> porphyrin structures with symmetrical substitution patterns show the nitrosyl ligand oriented about midway between a pair of Fe–N<sub>p</sub> vectors, giving dihedral angles ( $\phi$ , the angle between N–O and the closest Fe–N<sub>p</sub> bond) ranging from 38° to 41°, minimizing intramolecular contacts between pyrrolic nitrogens and nitrosyl oxygen. The one exception is [Fe(TPPBr<sub>4</sub>)(NO)],<sup>6</sup> found to have three conformations in two distinct crystalline forms, all of which possess nitrosyl groups oriented toward an Fe–N<sub>p</sub> bond of one of the trans bromo-substituted pyrrole rings (dihedral angles ( $\phi$ ) = 0°, 0°, and 18.4°). The bending of NO in a plane toward the less basic pyrrole rings<sup>8</sup> suggests there are NO orientational preferences with unsymmetrically substituted porphyrins. What are the nitrosyl orientation preferences in the asymmetrically substituted naturally occurring porphyrin derivatives?

The five-coordinate nitrosyl complexes [Fe(Por)(NO)], where Por = Deut, Meso, and Prot, have been prepared via reductive nitrosylation<sup>9</sup> of the iron(III) chlorides, but X-ray quality crystals of only [Fe(Deut)(NO)] have been obtained. The nitrosyl stretching frequencies,  $\nu(\text{NO})[\text{KBr}] = 1651, 1658, \text{ and } 1660 \text{ cm}^{-1}$  for [Fe(Deut)(NO)], [Fe(Meso)(NO)], and [Fe(Prot)(NO)], respectively, are lower than those previously observed for either OEP<sup>6</sup> or TPP<sup>10</sup> derivatives ( $\sim 1670\text{--}1680 \text{ cm}^{-1}$ ), yet comparable with that previously observed for the protoporphyrin derivative ( $\nu(\text{NO})[\text{KBr}] = 1655 \text{ cm}^{-1}$ ).<sup>11</sup>  $\nu(\text{CO})$  stretching frequencies of 1728–1740  $\text{cm}^{-1}$ , arising from the carbonyls of the propionic acid ester arms, are also observed.

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(3) The following abbreviations are used in this work: Deut, dianion of deuteroporphyrin IX dimethyl ester; Meso, dianion of mesoporphyrin IX dimethyl ester; OEP, dianion of octaethylporphyrin; oxoOEC, dianion of oxooctaethylchlorin (2-oxo-3,3',7,8,12,13,17,18-octaethylporphyrin); Prot, dianion of protoporphyrin IX dimethyl ester; TPP, dianion of meso-tetraphenylporphyrin; N<sub>p</sub>, a pyrrolic nitrogen. Abbreviations for all proteins are provided as part of the Supporting Information.

(4) Substituent pattern for the deuteroporphyrin IX dimethyl ester is Me, H, Me, H, Me, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Me, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Me, Me.

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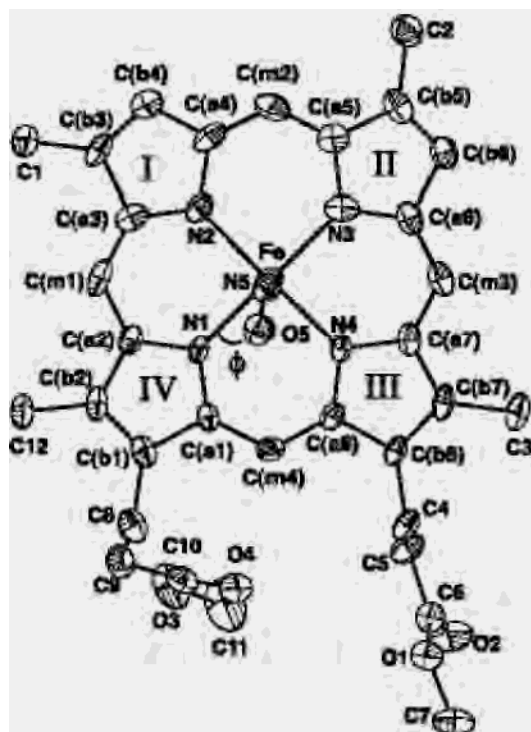
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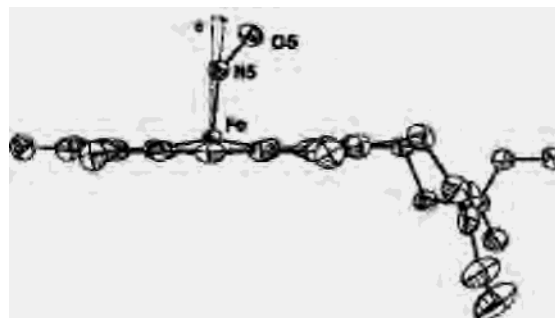


**Figure 1.** ORTEP diagram of [Fe(Deut)(NO)] illustrating 50% probability ellipsoids. The atom labeling scheme for the porphyrin core is shown. Hydrogen atoms are omitted for clarity. The dihedral angle ( $\phi$ ) and the pyrrole ring labeling scheme (I–IV) for deuteroporphyrin IX, protoporphyrin IX, and related porphyrins are illustrated.

The structure<sup>12</sup> shows an ordered nitrosyl ligand coordinated to an iron displaced 0.26 Å from the mean porphyrin plane which shows modest ruffling (Figure 1). The mean Fe–N<sub>p</sub> distance is 2.004 Å while the Fe–N(NO) distance of 1.723(3) Å is comparable with the distances in other five-coordinate {FeNO}<sup>7</sup> porphyrin complexes. The N–O distance of 1.187(4) Å and the Fe–N–O angle of 143.1(3)° are similar to those observed in other five-coordinate structures. The peripheral groups of the porphyrin display no disorder (Figure 2).

Off-axis tilting of the FeNO moiety occurs in several high-precision structures of both five-<sup>6</sup> and six-coordinate<sup>7</sup> {FeNO}<sup>7</sup> porphyrinates (Table 1). DFT calculations by Ghosh and Wondimagegn suggest that tilting leads to an increased overlap between NO and the iron porphyrinate orbitals.<sup>14</sup> The tilting of NO is accompanied by an asymmetry in the equatorial Fe–N<sub>p</sub> bonds, a short pair flanking the NO projection on the porphyrin plane and a longer pair opposite.

We observe a similar pattern of Fe–N<sub>p</sub> bonds and tilting of the {FeNO} moiety in the structure of [Fe(Deut)(NO)].



**Figure 2.** ORTEP diagram showing side view of [Fe(Deut)(NO)] illustrating 50% probability ellipsoids. Off-axis tilting ( $\theta$ ) of the Fe–N(NO) bond from the heme normal is shown.

The magnitude of this tilting (6.2°) and bond asymmetry are comparable with that observed in the related five-coordinate OEP derivatives (Table 1). Although the possible physiological significance of this ligand asymmetry remains unknown, the unsupported ligand tilting is clearly an intrinsic feature of nitrosyl-ligated iron(II) porphyrinates.

Figure 1 clearly shows that the nitrosyl ligand is oriented approximately midway between the Fe–N<sub>p</sub> bonds of pyrrole rings III and IV. The nitrosyl ligand therefore points toward the two slightly less basic pyrrole rings that bear the propionic acid ester groups, the direction expected if an orientational preference was to be found.<sup>8</sup> The nitrosyl orientation dihedral angle ( $\phi$ ) is 35° from the closest Fe–N<sub>p</sub> bond, a value slightly smaller than that observed for other five-coordinate {FeNO}<sup>7</sup> porphyrinates, except for the [Fe-(TPPBr<sub>4</sub>)(NO)] derivatives already noted.

The observation of these NO orientation preferences in model complexes led us to examine whether there was any pattern in the nitrosyl ligand orientation in heme proteins. Complete descriptions of nitrosyl ligand orientations in heme protein structures are frequently not available, but whenever possible, data was obtained from the published papers. If required, additional data has been derived from coordinates deposited in the Protein Data Bank.<sup>15</sup>

In order to completely describe the orientation of the NO ligand with respect to asymmetric porphyrin cores, we need to describe a more specific orientation angle ( $\phi^*$ )<sup>16</sup> in addition to the simple dihedral angle  $\phi$  that has been defined earlier. We use the standard ring numbering system shown in Figure 1 for all hemes. We use the signed torsion (dihedral) angle O–N–Fe–N<sub>IV</sub> ( $\phi^*$ )<sup>16</sup> where N<sub>IV</sub> is the nitrogen atom of pyrrole ring IV. We define the “top” of the porphyrin ring when the rotation of ring I → ring II is a clockwise rotation when viewed from the NO side; the value of the torsion angle is then the value of the clockwise rotation between the O–N–Fe and N–Fe–N<sub>IV</sub> planes. When the NO is on the “bottom”, all of these rotations will be anticlockwise and the angle  $\phi^*$  will have a negative sign.

The protein structures (Table 2)<sup>17</sup> show much larger ranges of nitrosyl bonding parameter values compared to the model

(12) X-ray quality crystals of [Fe(Deut)(NO)] were obtained by liquid diffusion of methanol into a chloroform solution of [Fe(Deut)(NO)] in sealed 8 mm tubes under an NO atmosphere. Crystal data: [Fe(Deut)(NO)], size = 0.28 × 0.24 × 0.21 mm, formula C<sub>32</sub>H<sub>32</sub>FeN<sub>5</sub>O<sub>5</sub>, triclinic, space group *P*1, *a* = 11.3518(14) Å, *b* = 11.6492(15) Å, *c* = 12.0065(15) Å,  $\alpha$  = 78.915(2)°,  $\beta$  = 77.590(2)°,  $\gamma$  = 64.663(2)°, *V* = 1392.4(3) Å<sup>3</sup>, *Z* = 2, *T* = 100 K, *D*<sub>calc</sub> = 1.485 cm<sup>3</sup>. Final *R* [*I* > 2 $\sigma$ (*I*): *R*<sub>1</sub> = 0.0594, *wR*<sub>2</sub> = 0.1460. Final *R* indices (all data): *R*<sub>1</sub> = 0.0738, *wR*<sub>2</sub> = 0.1543, GOF = 1.050.

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(16) We define  $\phi^*$  as the torsion angle for the four-atom unit O–N–Fe–N<sub>IV</sub>. This is the signed dihedral angle between the two planes incorporating the atoms O–N–Fe and N–Fe–N<sub>IV</sub> (nitrogen of pyrrole ring IV).

**Table 1.** Details of the  $N_4FeNO$  Geometry in Five-Coordinate  $\{FeNO\}^7$  Porphyrinate Derivatives<sup>a</sup>

complex	Fe–N(1) <sup>b</sup>	Fe–N(2) <sup>b</sup>	Fe–N(3) <sup>b</sup>	Fe–N(4) <sup>b</sup>	$\Delta(Fe-N_p)^{b,c}$	$\phi^{d,e}$	NO tilt ( $\theta$ ) <sup>e,f</sup>	ref
$\{FeNO\}^7$ Complexes								
[Fe(Deut)(NO)]	1.992(3)	<b>2.005(3)</b>	<b>2.035(3)</b>	1.987(3)	0.031	35.0	6.2	<i>j</i>
[Fe(OEP)(NO)] <sup>g</sup>	<b>2.016(2)</b>	1.989(2)	1.993(2)	<b>2.017(2)</b>	0.026	37.9	6.5	6
[Fe(OEP)(NO)] <sup>h</sup>	<b>2.0226(6)</b>	2.0000(6)	1.9985(6)	<b>2.0167(6)</b>	0.020	40.2	8.2	6
[Fe(oxoOEC)(NO)]	2.0174(13)	<b>2.0141(12)</b>	<b>2.0082(13)</b>	1.9974(12)	<i>i</i>	40.9	7.1	6
[Fe(OETAP)(NO)]	<b>1.938</b>	1.922	1.925	<b>1.941</b>	0.016	39.6	7.6	13

<sup>a</sup> Fe–N<sub>p</sub> bond distances given in bold are adjacent and expected to be long, see text. <sup>b</sup> Value in angstroms. <sup>c</sup> Difference between average Fe–N<sub>p</sub>(long) and Fe–N<sub>p</sub>(short). <sup>d</sup> Dihedral angle between Fe–N–O plane and closest Fe–N<sub>p</sub> vector. <sup>e</sup> Value in degrees. <sup>f</sup> Deviation of Fe–N(NO) bond from normal to porphyrin plane. <sup>g</sup> Monoclinic form. <sup>h</sup> Triclinic form. <sup>i</sup> Differences affected by presence of the oxopyrrole ring. <sup>j</sup> This work.

**Table 2.** Summary of Nitrosyl Geometry in Heme Proteins<sup>17</sup>

complex	Fe–N <sub>NO</sub> <sup>a</sup>	$\angle FeNO^b$	N–O <sup>a</sup>	$\phi^{b,c}$	$\phi^{*b,d}$
Iron(II) Five-Coordinate Hemes					
[Fe(Deut)(NO)]	1.723(3)	143.1(3)	1.187(4)	35	35
(T243N–P450nor)NO	1.9 <sup>e</sup>	131 <sup>e</sup>	1.45 <sup>e</sup>	41	–49
(cyt <i>c'</i> )NO (1) <sup>f</sup>	2.0	124	1.16 <sup>e</sup>	24	–114
(NP1)NO (I) <sup>g</sup>	<i>r</i>	125	<i>r</i>	44	134
(NP1)NO (II) <sup>h</sup>	<i>r</i>	135	<i>r</i>	45	135
(eNOS)NO(+4HB) <sup>i,j</sup>	1.80	160	1.15	44	136
(eNOS)NO(–4HB) <sup>i,k</sup>	1.80	160	1.14	34	146
( $\alpha$ -Hb)NO	1.74	145	1.1	25	245
(NP4)NO (I) <sup>l</sup>	2.0	110	<i>r</i>	1	269
(T243A–P450nor)NO	2.1 <sup>e</sup>	120 <sup>e</sup>	1.42 <sup>e</sup>	14	–284
(T243V–P450nor)NO	2.0 <sup>e</sup>	119 <sup>e</sup>	1.36 <sup>e</sup>	16	–286
(cyt <i>c'</i> )NO (2) <sup>m</sup>	2.0	132	1.17 <sup>e</sup>	38	–322
( $\alpha$ -T-Hb <sub>A</sub> )NO	1.74	150	1.13	<i>r</i>	<i>r</i>
Iron(II) Six-Coordinate Hemes					
( <i>cd</i> <sub>1</sub> -NIR( <i>tp</i> ))NO	2.0	131	1.3 <sup>e</sup>	30	120
(SiRHP)NO	1.76	125	1.17 <sup>e</sup>	40	–140
(hh-Mb)NO	2.03	147	1.14	~10	–190
(sw-L29F-Mb)NO	1.86 <sup>e</sup>	130	1.14 <sup>e</sup>	11	–191
(sw-Mb)NO	1.89	112	1.15	25	–205
( $\alpha$ -SNO-nitrosylHb <sub>A</sub> )NO	1.75	131	1.13	44	–224
( $\beta$ -SNO-nitrosylHb <sub>A</sub> )NO	1.74	123	1.11	34	–236
( $\beta$ -Hb)NO <sup>n</sup>	1.74	145	1.1	15	255
(fungal-NOR)NO	1.63	161	1.16	28	–298
(S286T-fungalNOR)NO	1.65	162	1.13	29	–299
(S286V-fungalNOR)NO	1.62	162	1.13	45	–315
(SHP)NO	1.8	112	1.36 <sup>e</sup>	45	315
( <i>cd</i> <sub>1</sub> -NIR( <i>pa</i> ))NO	1.8	135	1.15	26	334
(lupin-Lb <sup>ll</sup> )NO	1.72	147	1.355 <sup>e</sup>	2	358
( $\beta$ -T-Hb <sub>A</sub> )NO	1.74	155	1.12	<i>r</i>	<i>r</i>
(CCP)NO(1) <sup>o</sup>	1.82	135	<i>r</i>	<i>r</i>	<i>r</i>
(CCP)NO(2) <sup>p</sup>	1.82	125	<i>r</i>	<i>r</i>	<i>r</i>
(hh-Mb)NO <sup>q</sup>	1.76	150	1.12	<i>r</i>	<i>r</i>
(soybean-Lb <sup>ll</sup> )NO <sup>q</sup>	1.77	147	1.12	<i>r</i>	<i>r</i>

<sup>a</sup> Value in angstroms. <sup>b</sup> Value in degrees. <sup>c</sup> Dihedral angle between Fe–N–O plane and closest Fe–N<sub>p</sub> vector. <sup>d</sup> Signed torsion angle for the unit O–N–Fe–N<sub>p</sub> of pyrrole ring IV. Positive values refer to NO coordinated on the “top” of the porphyrin and represent a clockwise rotation about the N(NO)–Fe axis between the O–N–Fe and N–Fe–N<sub>p</sub> planes while negative values represent NO coordinated on the “bottom” and a counter-clockwise rotation is required. Absolute  $\phi^*$  values of 270°, 180°, and 90° result in NO orientations coincident with the Fe–N<sub>p</sub> bonds of pyrrole rings I, II, and III, respectively. <sup>e</sup> Value not reported in original literature report. Taken from PDB coordinates. <sup>f</sup> Nitrosyl conformer 1. <sup>g</sup> Two molecules in asymmetric unit, molecule I. <sup>h</sup> Two molecules in asymmetric unit, molecule II. <sup>i</sup> Values reported are averaged for both subunits. <sup>j</sup> Cofactor tetrahydrobiopterin present. <sup>k</sup> Cofactor tetrahydrobiopterin absent. <sup>l</sup> Two orientations of NO observed, orientation I. <sup>m</sup> Nitrosyl conformer 2. <sup>n</sup> Differs from  $\alpha$ -form by orientation of NO with respect to the heme and coordination number. <sup>o</sup> Disordered oxygen, position 1. <sup>p</sup> Disordered oxygen, position 2. <sup>q</sup> Structure determined by multiple scattering XAFS. <sup>r</sup> Not available.

complexes previously discussed. For example, the Fe–N–O angle ranges from 110° to 162°; the  $\{FeNO\}^7$  model

(17) An expanded version of Table 2 is available as Table S1 of the Supporting Information. The full table contains bibliographic information for each structure, a list of abbreviations, complete bond lengths and angles for the FeNO unit of each protein, and the orientation of the nitrosyl ligand with respect to the porphyrin core.

complexes display a much narrower range. Part of this difference is likely to arise from the lower resolution and inherent limitations of protein crystallography, although it is found that the structures determined at higher resolution display values closer to those of the model complexes. We therefore treat all protein structural data in a qualitative fashion, simply extracting a rough picture of the nitrosyl coordination in each system.

From the data of Table 2, we can make several observations about NO orientation in the heme proteins. First, coordinated NO is not limited to one particular face of the porphyrin. Second, the orientation of the nitrosyl with respect to the porphyrin core varies quite significantly. Both eclipsed and staggered orientations of the nitrosyl are observed. However, the projection of the NO onto the porphyrin plane is primarily found to be about midway between two Fe–N<sub>p</sub> vectors ( $\phi = 25$ – $45^\circ$ , 71%), suggesting that porphyrin–NO interactions have an effect in the protein environment. Third, NO orientations in all quadrants of the heme are observed, although there is only one example of NO orientation between pyrroles III and IV. Fourth, there is variation in NO ligand orientation between derivatives that might have been expected to have similar values, i.e., site-directed mutants vs native derivatives and two independent molecules in an asymmetric unit. This suggests that the constraints provided by the ligand binding pocket, although real, can be relatively soft. The steric constraints of both the porphyrin and ligand binding pocket contribute to the observed NO orientations in heme proteins. In summary, our structure of [Fe(Deut)(NO)] shows that the off-axis tilting of the FeNO moiety occurs in the naturally occurring porphyrin system. We see no reason to doubt that it may also occur in the protein systems. The other features of FeNO geometry in [Fe(Deut)(NO)] are comparable to those seen previously.

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**Supporting Information Available:** Figure S1 showing a formal diagram of the core of [Fe(Deut)(NO)] giving atomic displacements, in units of 0.01 Å, from the 24-atom mean plane; Table S1 giving FeNO bond lengths and angles for the relevant protein structures along with resolution, relative ligand orientation, expanded abbreviation list, and complete bibliographic information; Tables S2–S7 giving complete crystallographic details, atomic coordinates, bond distances and angles, anisotropic temperature factors, and fixed hydrogen atom positions for [Fe(Deut)(NO)] (PDF). The crystallographic information file (CIF) for [Fe(Deut)(NO)]. This material is available free of charge via the Internet at <http://pubs.acs.org>. IC034364E