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Models for Amide Ligation in Nonheme Iron Enzymes

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Lipoxygenase and isopenicillin N synthase belong to an emerging class of mononuclear nonheme iron(II) enzymes involved in dioxygen activation.[1] Though these two enzymes catalyze very different reactions, they possess common structural features. Crystal structures[2,3] of these enzymes (Figure 1) show the divalent metal centers to be coordinated to a 2-His-1-carboxylate facial triad[1c] as well as an amide ligand. These enzymes represent the first examples of amide ligation to iron in biology, a feature not anticipated by prior spectroscopic studies. The amide ligand appears to play a role in catalysis, as indicated by site-directed mutagenesis studies of the two enzymes.[4,5] To determine the effects of an amide ligand on the properties of an iron center, we have synthesized iron complexes of the tetradentate ligand bis(2-pyridylmethyl)-glycinamide (BPGm). $[Fe^{II}(BPGm)(O_2CCH_3)(CH_3OH)](BPh_4)$ (**1**) represents the first example of a complex with a ligand combination that corresponds closely to the coordination environments found for the metal centers in lipoxygenase and isopenicillin N synthase. For comparison, we also report the structures of $[Fe^{II}(TPA)(O_2CC(CH_3)_3)(CH_3OH)](BPh_4)$ (**2**, TPA = tris(2-pyridylmethyl)amine) and $[Fe^{III}_2(\mu\text{-}O)(\mu\text{-}O_2CCH_3)(BPGm)_2](ClO_4)_3$ (**3**) and their properties.

Anaerobic reaction of equimolar amounts of $Fe(ClO_4)_2$, BPGm,[6] and NaO_2CCH_3 in methanol at ambient temperature followed by the addition of $NaBPh_4$ afforded the yellow crystalline solid **1**.[7] The crystal structure[8] of the cation in **1** (Figure 2a) resembles that of the related **2**[9,10] (Figure 2b); both complexes have a mononuclear, distorted octahedral iron center bound to a tetradentate tripodal ligand, a monodentate carboxylate, and methanol. The metal centers are in the high-spin Fe(II) state as indicated by average Fe−N distances of 2.22 Å and their isotropically shifted ^1H NMR signals (see Supporting Information). The amide function in **1** binds to the iron through its carbonyl oxygen atom with an $Fe-O_{amide}$ distance of 2.170(5) Å),[11] which is slightly longer than those observed in Fe(II)−DMF complexes (average 2.12 Å).[12] However it is significantly longer than those found for the terminal carboxylates in **1** (2.024(5) Å) and **2** (1.988(4) Å), which probably reflects the difference in charge between the amide and carboxylate groups. From this perspective, the amide ligand behaves more like a pyridine, as indicated by the comparable length of the corresponding $Fe-N_{pyridine}$ bond in **2** (2.212(6) Å) when the differing covalent radii of oxygen and nitrogen are taken into account.

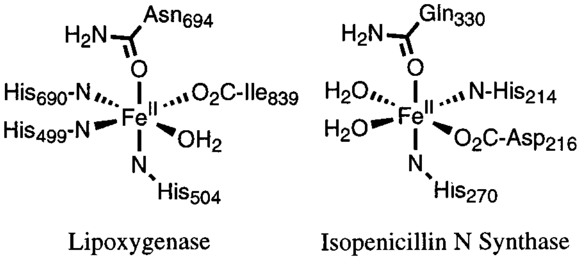

Figure 1. Active sites of lipoxygenase and isopenicillin N synthase.

The near-IR spectra of **1** and **2** in CH_3CN can be compared to assess the ligand field strength of the amide function. Complex **1** exhibits a broad feature with λ_{max} at 1000 nm (ϵ = 8 M^{-1} cm^{-1}), which can be attributed to the $^5T_{2g} \rightarrow {}^5E_g$ transition of an octahedral d^6 ion. The corresponding band for **2** has a λ_{max} at 956 nm (ϵ = 7 M^{-1} cm^{-1}), the blue shift indicating that the amide in BPGm exerts a weaker ligand field than the corresponding pyridine on TPA. This result is consistent with a comparative study of soybean and mammalian lipoxygenases,[13] whose active sites differ by the substitution of the amide ligand in the former with a histidine in the latter.

A further assessment of the effects of an amide group can be obtained from the corresponding (μ-oxo)diiron(III) complex. Exposure of **1** to O_2 results in the formation of **3**,[14] whose crystal structure[15] (Figure 2c) shows a (μ-oxo)diiron(III) unit supported by an acetate bridge. The dimensions of this dibridged core resemble those of the closely related complexes $[Fe_2(\mu\text{-}O)(\mu\text{-}$

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(1) (a) Que, L., Jr.; Ho, R. Y. N. *Chem. Rev.* **1996**, *96*, 2607−2624. (b) Feig, A. L.; Lippard, S. J. *Chem. Rev.* **1994**, *94*, 759−805. (c) Hegg, E. L.; Que, L., Jr. *Eur. J. Biochem.*, in press.

(2) Minor, W.; Steczko, J.; Stec, B.; Otwinowski, Z.; Bolin, J. T.; Walter, R.; Axelrod, B. *Biochemistry* **1996**, *35*, 10687−10701.

(3) (a) Roach, P. L.; Clifton, I. J.; Fülöp, V.; Harlos, K.; Barton, G. J.; Hajdu, J.; Andersson, I.; Schofield, C. J.; Baldwin, J. E. *Nature* **1995**, *375*, 700−704. (b) Roach, P. L.; Clifton, I. J.; Hensgens, C. M. H.; Shibata, N.; Schofield, C. J.; Hajdu, J.; Baldwin, J. E. *Nature* **1997**, *387* 827−830.

(4) Kramer, J. A.; Johnson, K. R.; Dunham, K. R.; Sands, R. H.; Funk, M. O., Jr. *Biochemistry* **1994**, *33*, 15017−15022.

(5) (a) Landman, O.; Borovok, I.; Aharanowitz, Y.; Cohen, G. *FEBS Lett.* **1997**, *405*, 172−174. (b) Sami, M.; Brown, T. J. N.; Roach, P. L.; Schofield, C. J.; Baldwin, J. E. *FEBS Lett.* **1997**, *405*, 191−194.

(6) The BPGm ligand was prepared by refluxing equimolar amounts of bis(2-pyridylmethyl)amine and bromoacetamide and excess Na_2CO_3 in CH_3CN for 2 h and isolated as the HBr salt. After neutralalization and extraction with CH_2Cl_2, the ligand was obtained in 85% yield.

(7) Anal. Calcd for $[Fe^{II}(BPGm)(O_2CCH_3)(MeOH)](BPh_4)\cdot CH_3OH\cdot 3H_2O$: C, 62.40; H, 6.56; N, 6.93. Found: C, 62.50; H, 5.84; N, 7.05.

(8) Crystal data for **1**·$2CH_3OH$: yellow needles, space group $P2_1/c$ (No. 14), with a = 17.4278(13) Å, b = 9.0809(7) Å, c = 27.361(2) Å, β = 105.936(2)°, V = 4163.7(5) $Å^3$, and Z = 4. The structure was refined by using 2385 reflections ($I > 3\sigma(I)$) and 374 parameters to final discrepancy indices R_1 = 0.0678 and wR_2 = 0.1243, based on F^2 derivatives.

(9) Anal. Calcd for $[Fe^{II}(TPA)(O_2CC(CH_3)_3)](BPh_4)$: C, 73.62; H, 6.13; N, 7.31. Found: C, 73.90; H, 6.20; N, 7.05.

(10) Crystal data for **2**·CH_3OH: space group $P2_12_12_1$ (No. 19) with a = 9.4635(2) Å, b = 23.8909(4) Å, c = 39.7436(4) Å, V = 8985.7(3) $Å^3$, and Z = 8. The structure was solved by using 8740 reflections ($I > 2\sigma(I)$) and 1068 parameters to final discrepancy indices R_1 = 0.0634 and wR_2 = 0.1278, based on F^2 derivatives. There were two independent molecules of **2** in the crystallographic asymmetric unit.

(11) The oxygen and nitrogen atoms of the amide ligand were assigned in the structure shown by a number of criteria: the comparable sizes of the thermal ellipsoids, the C−X bond lengths, and the presence of a hydrogen-bonding network for the NH_2 group.

(12) (a) Constant, G.; Daran, J. C.; Jeannin, Y. *J. Inorg. Nucl. Chem.* **1971**, *33*, 4209−4217. (b) Müller, A.; Schladerbeck, N. H.; Krickemeyer, E.; Bögge, H.; Schmitz, K. *Z. Anorg. Allg. Chem.* **1989**, *570*, 7−36.

(13) Pavlosky, M. A.; Zhang, Y.; Westre, T. E.; Gan, Q.-F.; Pavel, E. G.; Campochiaro, C.; Hedman, B.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1995**, *117*, 4316−4327.

(14) Alternatively, **3** can also be obtained from $Fe(ClO_4)_3$, BPGm, and NaO_2CCH_3 in a 2:2:1 ratio in methanol in 90% yield. Anal. Calcd for $[Fe^{III}_2(\mu\text{-}O)(\mu\text{-}O_2CCH_3)(BPGm)_2](ClO_4)_3$: C, 36.11; H, 3.51; N, 11.23. Found: C, 35.86; H, 3.56; N, 10.94. **Caution!** Metal complexes with organic ligands and perchlorate anions are potentially explosive.

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Table 1. Comparison of the Properties of $[Fe^{II}(L)(O_2CR)(CH_3OH)]$ and $[Fe^{III}_2(O)(L)_2(O_2CR)]$ Complexes

L[a]	$r(Fe^{II}-X)$ (Å)	λ_{max} (nm)	$r(Fe^{III}-X)$ (Å)	λ_{max} (nm)	$E_{1/2}^{red}$ (mV vs NHE)
TPA (X = py)	2.212(6)	956	2.116(6)[b]	700, 1050[b]	+150 (rev)[c]
BPGm (X = C(O)NH$_2$)	2.170(5)	1000	2.043(5)	645, 950	−200 (irrev)
BPG (X = COO$^-$)	[2.024(5)][d]		1.988(5)[e]	636, 920[e]	−350 (irrev)

[a] X is the variable pendant arm on the tripodal ligand. [b] From ref 16. [c] From ref 20. [d] We have not been able to synthesize the corresponding mononuclear BPG complex; we approximate this value based on the Fe−O_2CR distance observed in **1**. [e] From ref 17.

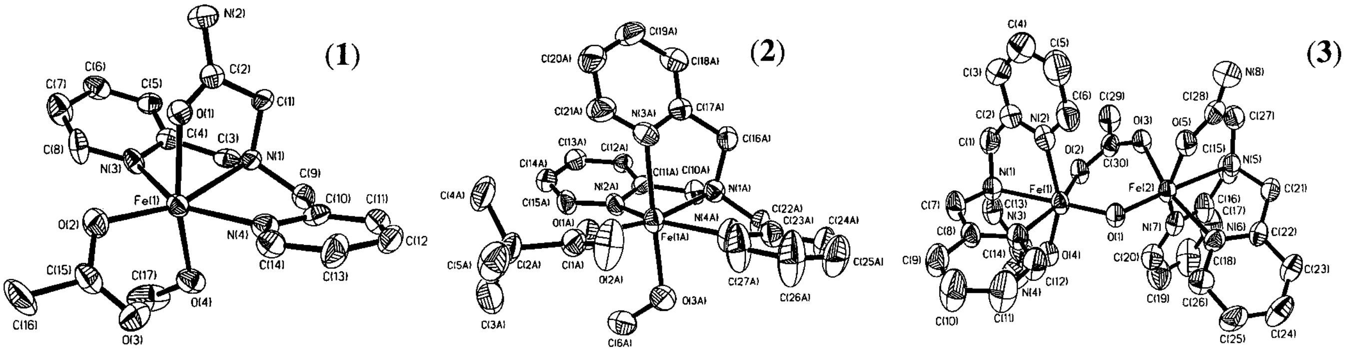

Figure 2. Crystal structures of the cation in $[Fe(CH_3CO_2)(BPGm)(MeOH)](BPh_4)$ (**1**), $[Fe(TPA)(O_2CC(CH_3)_3)(CH_3OH)](BPh_4)$ (**2**), and $[Fe_2(\mu\text{-}O)(\mu\text{-}O_2CCH_3)(BPGm)_2](ClO_4)_3$ (**3**). Selected interatomic distances and an angle are as follows. For **1**: Fe(1)−O(1) 2.185(6) Å, Fe(1)−O(2) 2.035(5) Å, Fe(1)−O(4) 2.132(6) Å, Fe(1)−N(1) 2.288(7) Å, Fe(1)−N(3) 2.208(7) Å, Fe(1)−N(4) 2.184(7) Å. For **2**: Fe(1A)−O(1A) 1.988(4) Å, Fe(1A)−O(3A) 2.154(4) Å, Fe(1A)−N(1A) 2.264(5) Å, Fe(1A)−N(2A) 2.207(5) Å, Fe(1A)−N(3A) 2.212(6) Å, Fe(1A)−N(4A) 2.207(5) Å. For **3**: Fe(1)−O(1) 1.800(4) Å, Fe(1)−O(2) 2.063(4) Å, Fe(1)−O(4) 2.045(5) Å, Fe(1)−N(1) 2.252(5) Å, Fe(1)−N(2) 2.126(6) Å, Fe(1)−N(3) 2.109(5) Å, Fe(2)−O(1) 1.783(4) Å, Fe(2)−O(3) 2.053(4) Å, Fe(2)−O(5) 2.042(5) Å, Fe(2)−N(5) 2.240(5) Å, Fe(2)−N(6) 2.125(5) Å, Fe(2)−N(7) 2.139(6) Å, Fe(1)···Fe(2) 3.202(1) Å, Fe(1)−O(1)−Fe(2) 126.6(2)°.

$O_2CCH_3)(TPA)_2](ClO_4)_3$ (**4**)[16] and $[Fe_2(\mu\text{-}O)(\mu\text{-}O_2CC_6H_5)(BPG)_2](ClO_4)$ (**5**)[17] (BPG = bis(2-pyridylmethyl)glycinate), with Fe−Fe distances of 3.22 ± 0.02 Å and Fe−O−Fe angles of 126−130°. The remaining sites of the iron ions in **3** are occupied by the BPGm ligand, with the amine nitrogens positioned trans to the oxo group as found in **5**. The amide in **3** binds trans to one of the pyridines with an Fe−O_{amido} bond of 2.04 Å, comparable in length to that found for an Fe^{III}−DMF complex[18] (2.03(1) Å) but much shorter than that of the other recently reported BPGm complex, $[Fe_2(\mu\text{-}O)Cl_2(BPGm)_2](ClO_4)_2$ (2.165(6) Å),[19] where the amido oxygens are trans to the oxo bridge. Unlike in the Fe(II) complexes, the Fe−O_{amide} bond in **3** is only 0.05 Å longer than the terminal Fe−$O_{carboxylate}$ bond in **5** and is comparable in length to the Fe−O bonds of the bridging carboxylate. This comparison suggests that the interaction of the amide with the Fe(III) center makes it behave more like a carboxylate ligand.

The optical and electrochemical properties of **3** support the notion that the amide ligand in **3** is more carboxylate-like. The optical spectrum of **3** in CH_3CN contains features with λ_{max} (ϵ, M^{-1} cm^{-1}) = 411 (2100), 435 (1700), 479 (800), 489 (1000), 521 (320), 645 (180), and 950 (8) nm, which are very similar to those observed for **4** and **5.** The bands in the 550−800 and 800−1100 nm spectral regions can be assigned to the $^6A_1 \rightarrow {}^4T_2$ and $^6A_1 \rightarrow {}^4T_1$ ligand field transitions of a d^5 ion, respectively,[21] and spectral shifts reflect changes in ligand field strength for complexes with similar Fe−O−Fe angles. For high-spin d^5 ions, increasing ligand field strength results in transitions of lower energy. From Table 1 it can be seen that the ligand field bands of **3** approach those of **5** and differ significantly in energy from those of **4**. Similarly, the redox properties of **3** resemble those of **5**. Both **3** and **5** exhibit irreversible one-electron reductions, at −200 and −350 mV vs NHE, respectively, while **4** exhibits a quasireversible reduction at +150 mV.[20] Taken together, the data suggest that the amide group acts like a carboxylate group with a partial negative charge; this may come about by greater population of the imino form of the amide as a result of its interaction with the Lewis acidic Fe^{III} center.

In conclusion, we have prepared and characterized Fe^{II} and Fe^{III} complexes of BPGm, a tripodal ligand with a pendant amide group to model the coordination environments of the iron centers in lipoxygenase and isopenicillin N synthase. By comparison with structurally related complexes having pendant carboxylate and pyridine ligands, the amide ligand appears to behave like a neutral pyridine ligand in the Fe^{II} complex but becomes more like a carboxylate with a partial negative charge when the iron center becomes Fe^{III}. This ability of the amide ligand to modulate its electronic effects may be useful for tuning the properties of an iron center as it goes through a redox cycle.

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Supporting Information Available: ^1H NMR spectra of **1** and **2** (2 pages). X-ray crystallographic files, in CIF format, for complexes **1**−**3** are available on the Internet only. Ordering and access information is given in any current masthead page.

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(15) Crystal data for **3**·$2CH_3CN$: space group $P2_1/n$ (No. 14) with a = 13.6381(2) Å, b = 23.5590(3) Å, c = 15.7021(2) Å, β = 108.594(1)°, V = 4781.7(8) Å^3, and Z = 4. The structure was solved by using 5609 reflections ($I > 2\sigma(I)$) and 650 parameters to final discrepancy indices R_1 = 0.0822 and wR_2 = 0.2132, based on F^2 derivatives.

(16) Norman, R. E.; Yan, S.; Que, L., Jr.; Backes, G.; Ling, J.; Sanders-Loehr, J.; Zhang, J. H.; O'Connor, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 1554−1562.

(17) Ménage, S.; Que, L., Jr. *New J. Chem.* **1991**, *15*, 431−438.

(18) Reiff, W. M.; Witten, E. H.; Mottle, K.; Brennan, T. F.; Garafalo, A. R. *Inorg. Chim. Acta* **1983**, *77*, L27-L30.

(19) Ito, S.; Okuno, T.; Matsushima, H.; Tokii, T.; Nishida, Y. *J. Chem. Soc., Dalton Trans.* **1996**, 4479−4484.

(20) Holz, R. C.; Elgren, T. E.; Pearce, L. L.; Zhang, J. H.; O'Connor, C. J.; Que, L., Jr. *Inorg. Chem.* **1993**, *32*, 5844−5850.

(21) Reem, R. C.; McCormick, J. M.; Richardson, D. E.; Devlin, F. J.; Stephens, P. J.; Musselman, R. L.; Solomon, E. I. *J. Am. Chem. Soc.* **1989**, *111*, 4688−4704.