# C−H Bond Activation by a Ferric Methoxide Complex: A Model for the Rate-Determining Step in the Mechanism of Lipoxygenase

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Lipoxygenases (LOs) are mononuclear, non-heme iron enzymes found in both plants and animals that catalyze the oxidative conversion of 1,4-diene-containing fatty acids to alkyl hydroperoxides.[1,2] The rate-determining step (RDS) of the mechanism is generally accepted to be a hydrogen atom abstraction (HA) of the weak, substrate C−H bond (∼77 kcal/mol)[3] to generate a substrate-radical which is subsequently trapped by dioxygen.[4,5] The species postulated to be responsible for the HA is a ferric−hydroxide complex, which is subsequently reduced by the H-atom to a ferrous−water complex (eq 1).[6] The reactivity of LOs under anaerobic conditions are

$$Fe^{III}-OH + \underset{R}{\overset{H\,H}{\diagup\!\diagdown}}R' \longrightarrow Fe^{II}-H_2O + \underset{R}{\overset{H}{\diagup\!\diagdown}}R' \qquad (1)$$
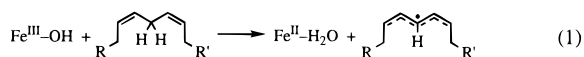
consistent with this mechanism as substrate reduces the active ferric site and generates alkyl-radicals.[7,8] However, this mechanism contrasts with traditional biological mechanisms which are generally thought to proceed through a high-valent iron−oxo or ferric−peroxy species.[9,10] Presented here is an example of C−H bond HA by a ferric−methoxide complex providing chemical precedent for the proposed RDS of LOs. Additionally, the properties of the substrates and products predict that this reaction is thermodynamically favorable. The chemical similarities of this model complex to the iron-sites in LOs strongly support a HA by a ferric−hydroxide species.

The iron coordination environment in LOs generates an iron center with a very positive Fe(III)/Fe(II) reduction potential (∼0.6 V vs SHE).[11] X-ray structural analysis and spectroscopy suggest that 6-coordinate ferrous and ferric forms of soybean-1 LO have a single exogenous water and hydroxide ligand, respectively.[12−16] Of the remaining five endogenous ligands, only one is anionic, which is consistent with the positive redox

Scheme 1
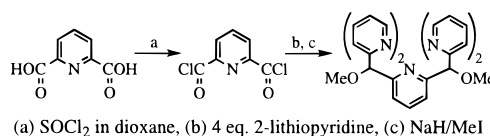
(a) SOCl₂ in dioxane, (b) 4 eq. 2-lithiopyridine, (c) NaH/MeI

potential. A short iron−ligand bond distance observed in the ferric form is attributed to a hydroxide ligand.[15] Reduction of this species results in little change in the coordination environment beyond lengthening a single iron−ligand bond distance.

A new ligand has been designed and synthesized (Scheme 1) to mimic specific attributes of the iron coordination site in LOs, namely a 5-coordinate environment which can accommodate a sixth exogenous solvent ligand. The ligand, 2,6-bis-((2-pyridyl)methoxymethane)pyridine (**PY5**), is composed of five pyridine subunits and accommodates a single metal in a nearly idealized square pyramidal coordination. A 6-coordinate ferrous complex is isolated directly from a 1:1 mixture of Fe-(OTf)₂ and **PY5** as a methanol solvate: [Fe(**PY5**)(MeOH)]-(OTf)₂ (**1**).[17] As in the resting state forms of the various plant and animal LOs,[18,19] the complex is air-stable and high-spin.[20] The structure of **1**·MeOH[21,22] reveals a 6-coordinate ferrous cation bound to all five of the pyridyl subunits and an exogenous methanol ligand. The Fe−O bond length of 2.04 Å is notably short for methanol bound to a divalent metal. The oxidation potential of +0.930 V (in methanol vs SHE),[21] is similar to other ferrous complexes composed of neutral nitrogen atom ligands.[23,24]

Oxidation of **1** with 0.5 equiv of either H₂O₂ or iodosobenzene in MeOH yields the stable ferric complex [Fe(**PY5**)(OMe)]-(OTf)₂ (**2**). The structure of **2**·MeOH[21,25] (Figure 1) reveals a coordination environment similar to that found in **1** (RMS error = 0.21 Å), although the metals differ in oxidation state. Two large structural changes occur upon oxidation of **1** to **2**: the Fe−O bond length is dramatically decreased from 2.04 (**1**) to 1.78 (**2**) Å and the Fe−O−CH₃ angle is significantly opened from 134° (**1**) to 165° (**2**). This short bond length and large angle are the extremes for terminally bound methoxides in structurally characterized iron complexes[26] and have only been observed with more electron-deficient metals such as titanium[27] and zirconium.[28] These structural parameters of **2** are consistent with multiple-bond character of the Fe−OMe bond, similar to that proposed to occur in μ-oxo−ferric dimer complexes.[29] The neutral **PY5** ligation to a ferric center generates an extremely

(1) Samuelsson, B.; Dahlén, S.-E.; Lindgren, J. Å.; Rouzer, C. A.; Serhan, C. N. *Science* **1987,** *237,* 1171.

(2) Gardner, H. W. *Biochim. Biophys. Acta* **1991,** *1084,* 221.

(3) Clark, K. B.; Culshaw, P. N.; Griller, D.; Lossing, F. P.; Simões, J. A. M.; Walton, J. C. *J. Org. Chem.* **1991,** *56,* 5535.

(4) de Groot, J. J. M. C.; Veldink, G. A.; Vliegenthart, J. F. G.; Boldingh, J.; Wever, R.; van Gelder, B. F. *Biochim. Biophys. Acta* **1975,** *377,* 71.

(5) Schilstra, M. J.; Veldink, G. A.; Vliegenthart, J. F. G. *Biochemistry* **1994,** *33,* 3974.

(6) Nelson, M. J. *J. Am. Chem. Soc.* **1988,** *110,* 2985.

(7) de Groot, J. J. M. C.; Garssen, G. J.; Vliegenthart, J. F. G.; Boldingh, J. *Biochim. Biophys. Acta* **1973,** *326,* 279.

(8) Nelson, M. J.; Cowling, R. A.; Seitz, S. P. *Biochemistry* **1994,** *33,* 4966.

(9) Que, L., Jr.; Ho, R. Y. N. *Chem. Rev.* **1996,** *96,* 2607.

(10) Sono, M.; Roach, M. P.; Coulter, E. D.; Dawson, J. H. *Chem. Rev.* **1996,** *96,* 2841.

(11) Nelson, M. J. *Biochemistry* **1988,** *27,* 4273.

(12) Boyington, J. C.; Gaffney, B. J.; Amzel, L. M. *Science* **1993,** *260,* 1482.

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

(14) Van der Heijdt, L. M.; Feiters, M. C.; Navaratnam, S.; Nolting, H.-F.; Hermes, C.; Veldink, G. A.; Vliegenthart, J. F. G. *Eur. J. Biochem.* **1992,** *207,* 793.

(15) Scarrow, R. C.; Trimitsis, M. G.; Buck, C. P.; Grove, G. N.; Cowling, R. A.; Nelson, M. J. *Biochemistry* **1994,** *33,* 15023.

(16) 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.

(17) OTf = CF₃SO₃⁻.

(18) Slappendel, S.; Malmström, B. G.; Petersson, L.; Ehrenberg, A.; Veldink, G. A.; Vliegenthart, J. F. G. *Biochem. Biophys. Res. Commun.* **1982,** *108,* 673.

(19) Petersson, L.; Slappendel, S.; Vliegenthart, J. F. G. *Biochim. Biophys. Acta* **1985,** *828,* 81.

(20) Solution magnetic susceptibilities are μ_eff (**1**) = 4.7 μ_B and μ_eff (**2**) = 5.1 μ_B at 297 K in methanol as measured by the Evans method.

(21) See the Supporting Information.

(22) Crystal data for **1** at 203 K: triclinic, P1̄ (No. 2), a = 12.417(2) Å, b = 12.672(2) Å, c = 13.345(1) Å, α = 80.00(1)°, β = 63.509(9)°, γ = 89.51(1)°, V = 1845.1(5) Å, Z = 2. On the basis of 5613 unique reflections (I > 3.00σ(I)) and 514 variable parameters, R (R_w) = 0.053 (0.074).

(23) Bernal, I.; Jensen, I. M.; Jensen, K. B.; McKenzie, C. J.; Toftlund, H.; Tuchagues, J.-P. *J. Chem. Soc., Dalton Trans.* **1995,** 3667.

(24) Koch, W. O.; Barbieri, A.; Grodzicki, M.; Schünemann, V.; Trautwein, A. X.; Krüger, H.-J. *Angew. Chem., Int. Ed. Engl.* **1996,** *35,* 422.

(25) Crystal data for **2** at 203 K: triclinic, P1̄ (No. 2), a = 9.532(2) Å, b = 9.966(1) Å, c = 19.852(3) Å, α = 96.17(1)°, β = 100.12(2)°, γ = 92.53(2)°, V = 1841.8(5) Å, Z = 2. On the basis of 4593 unique reflections (I > 3.00σ(I)) and 597 variable parameters, R (R_w) = 0.059 (0.062).

(26) (a) Hoard, J. L.; Hamor, M. J.; Hamor, T. A.; Caughey, W. S. *J. Am. Chem. Soc.* **1965,** *87,* 2312. (b) Lecomte, C.; Chadwick, D. L.; Coppens, P.; Stevens, E. D. *Inorg. Chem.* **1983,** *22,* 2982. (c) Hatano, K.; Uno, T. *Bull. Chem. Soc. Jpn.* **1990,** *63,* 1825. (d) Holman, T. R.; Andersen, K. A.; Anderson, O. P.; Hendrich, M. P.; Juarez-Garcia, C.; Münck, E.; Que, L., Jr. *Angew. Chem., Int. Ed. Engl.* **1990,** *29,* 921.
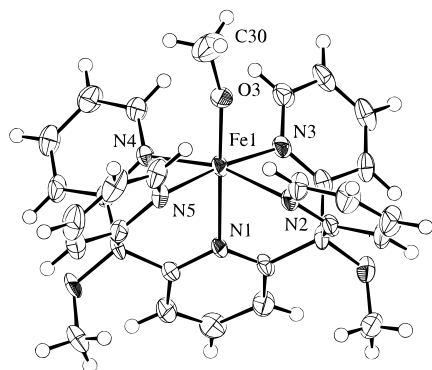
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Figure 1. ORTEP drawing of the cation of **2** with atom labeling scheme. Selected bond lengths (Å) and bond angles (deg) are as follows: Fe1−N1, 2.147(4); Fe1−N2, 2.154(4); Fe1−N3, 2.178(4); Fe1−N4, 2.223(4); Fe1−N5, 2.107(4); Fe1−O3, 1.782(3); O3−C30, 1.375(7); C30−O3−Fe1, 164.9(4).
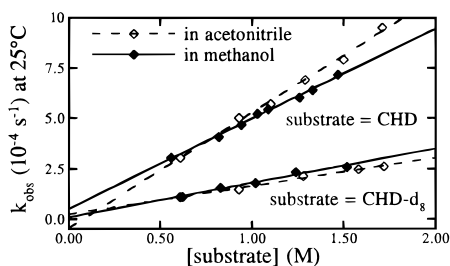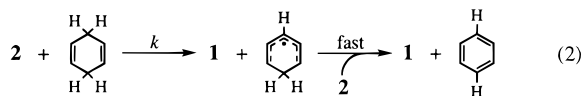

Figure 2. Plot of observed rate of decay of **2** (1.25 mM) with substrate.

Lewis acidic metal center. The short Fe−OMe (1.78 Å) distance is reminiscent of the short Fe−O distance (1.88 Å) observed in the proposed ferric−hydroxide species in activated LOs.[15] The aerobic and thermal stability of **2** is intriguing, as no other ferric complex with **PY5** has been successfully isolated. Solution magnetic susceptibility measurements at 298 K show this complex to be high-spin,[20] similar to activated LOs. The reduction potential occurs at +0.660 V (in methanol vs SHE),[22] a potential similar to the ferric center of soybean-1 LO.

A substrate model for the weak C−H bonds is 1,4-cyclo-hexadiene (CHD) which has a C−H bond dissociation energy (BDE) of 73 kcal/mol.[30] CHD reduces **2** to **1** with the formation of benzene[31] in a reaction consistent with second order kinetics (eq 2). As measured in both methanol and acetonitrile, the

$$ \mathbf{2} + \underset{\substack{H\ H}}{\underset{\substack{H\ H}}{\bigcirc}} \xrightarrow{k} \mathbf{1} + \underset{\substack{H\ H}}{\bigcirc} \xrightarrow[\mathbf{2}]{fast} \mathbf{1} + \underset{H}{\bigcirc} \qquad (2) $$

decay of **2** follows first-order kinetics under conditions of excess substrate, and the rate constant is linearly dependent on the substrate concentration (Figure 2). The second-order rate law is $d[\mathbf{1}]/dt = k[\mathbf{2}][\text{substrate}]$,[32] with a k of $4.5(\pm 0.2) \times 10^{-4}$ s^{-1}

Scheme 2

		energy (kcal/mol) in MeOH
$[\text{Fe}^{III}(\mathbf{PY5})(\text{OMe})]^{2+} + e^-$ ⟶ $[\text{Fe}^{II}(\mathbf{PY5})(\text{OMe})]^{1+}$	(E_{pc} = 0.660 V)	-15
$[\text{Fe}^{II}(\mathbf{PY5})(\text{OMe})]^{1+} + H^+$ ⟶ $[\text{Fe}^{II}(\mathbf{PY5})(\text{MeOH})]^{1+}$	(pK_a = 9.7)	-13
$H^\bullet$ ⟶ $^1/_2 H_2$		} −56[39]
$^1/_2 H_2$ ⟶ $H^+ + e^-$		
$[\text{Fe}^{III}(\mathbf{PY5})(\text{OMe})]^{2+} + H^\bullet$ ⟶ $[\text{Fe}^{II}(\mathbf{PY5})(\text{MeOH})]^{1+}$	−BDE (O−H)	-84

in methanol and $5.7(\pm 0.4) \times 10^{-4}$ s^{-1} in acetonitrile. Isotope effects ($k_{\text{C6H8}}/k_{\text{C6D8}}$) of 2.7 (MeOH) and 4.0 (CH$_3$CN) are observed[33] when using the perdeuterated analog of CHD.[34] Thus, C−H bond scission is associated with the RDS of the reaction of the ferric complex with the substrate. The similar rate constants in both protic (MeOH) and aprotic (CH$_3$CN) solvents are consistent with a mechanism of HA.[35]

Thermodynamic considerations predict that for CHD to reduce **2** to **1**, the energy of the O−H bond created in **1** must be similar to the energy of the cleaved substrate C−H bond. The O−H bond strength of the ligated methanol adduct in **1** is estimated as 84 kcal/mol in methanol from the reduction potential of **2** (0.660 V) and the proton affinity of **1** (pK_a = 9.7)[36] in methanol (Scheme 2).[37−39] This suggests that **2** is thermodynamically competent to abstract a H-atom from any substrate with a C−H BDE ≈ 84 kcal/mol. Indeed, a linear correlation between log k and the C−H BDE of effective reducing substrates is consistent with a HA mechanism.[38,40,41]

A new pentadentate ligand, **PY5**, has been designed and synthesized to mimic the Lewis acidic iron site in LOs. The ferric−methoxide complex of **PY5** is reduced by substrates with weak C−H bonds to yield the ferrous−methanol complex in a process most consistent with HA from substrate. This provides not only the first chemical precedent for the proposed RDS in the mechanism of LOs but also a thermodynamic rational of why this reaction is favorable.

(27) (a) Wright, D. A.; Williams, D. A. *Acta Crystallogr., Sect. B: Struct. Sci.* **1968**, *24*, 1107. (b) Boreham, C. J.; Buisson, G.; Duee, E.; Jordanov, J.; Latour, J.-M.; Marchon, J.-C. *Inorg. Chim. Acta* **1983**, *70*, 77. (c) Aslan, H.; Sielisch, T.; Fischer, R. D. *J. Organomet. Chem.* **1986**, *315*, C69. (d) Bodner, A.; Jeske, P.; Weyhermüller, T.; Wieghardt, K.; Dubler, E.; Schmalle, H.; Nuber, B. *Inorg. Chem.* **1992**, *31*, 3737. (e) Jeske, P.; Haselhorst, G.; Weyhermüller, T.; Wieghardt, K.; Nuber, B. *Inorg. Chem.* **1994**, *33*, 2462.
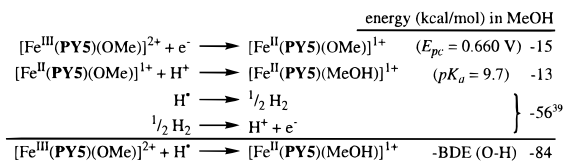
(28) Karia, R.; Willey, G. R.; Drew, M. G. B. *J. Chem. Soc., Dalton Trans.* **1986**, 2493.

(29) La Mar, G. N.; Eaton, G. R.; Holm, R. H.; Walker, F. A. *J. Am. Chem. Soc.* **1973**, *95*, 63.

(30) Burkey, T. J.; Majewski, M.; Griller, D. *J. Am. Chem. Soc.* **1986**, *108*, 2218.

(31) The yield of benzene in a 1:1 reaction of CHD and **2** under anaerobic conditions is nearly 50%.

(32) In order to account for the reduction of 2 equiv of **2** in the formation of 1 equiv of benzene (eq 2), the value of [**2**] used to calculate the rate constant is half that of the initial concentration.

(33) These isotope effects are significantly different from that observed in LO (k_H/k_D > 50). See references in the following: Glickman, M. H.; Klinman, J. P. *Biochemistry* **1995**, *34*, 14077.

(34) Brandsma, L.; van Soolingen, J.; Andringa, H. *Synth. Commun.* **1990**, *20*, 2165.

(35) Avila, D. V.; Brown, C. E.; Ingold, K. U.; Lusztyk, J. *J. Am. Chem. Soc.* **1993**, *115*, 466.

(36) The pK_a of **1** in methanol was spectrophotometrically determined using bases with known pK_a values in methanol. Rochester, C. H.; Rossall, B. *J. Chem. Soc. B* **1967**, 743.

(37) Bordwell, F. G.; Zhang, X.-M.; Satish, A. V.; Cheng, J.-P. *J. Am. Chem. Soc.* **1994**, *116*, 6605.

(38) Gardner, K. A.; Mayer, J. M. *Science* **1995**, *269*, 1849.

(39) The sum of the reduction potential and BDE of H$_2$ in methanol has been calibrated in methanol to be −56 kcal/mol using phenol.

(40) Two additional substrates with greater C−H bond strengths, 9,10-dihydroanthracene (BDE = 78 kcal/mol)[40a] and 2,3-dimethyl-2-butene (BDE = 82 kcal/mol),[40b] show similar kinetic profiles to that of CHD with **2**, with rate constants in methanol of 1.2×10^{-4} and 0.25×10^{-4} M^{-1} s^{-1}, respectively. (a) Bordwell, F. G.; Cheng, J.-P.; Ji, G.-Z.; Satish, A. V.; Zhang, X. *J. Am. Chem. Soc.* **1991**, *113*, 9790. (b) Luo, Y.-R.; Holmes, J. L. *J. Phys. Chem.* **1994**, *98*, 303.

(41) Cook, G. K.; Mayer, J. M. *J. Am. Chem. Soc.* **1995**, *117*, 7139.