Hydrogen Transfer Reactivity of a Ferric Bi-imidazoline Complex That Models the Activity of Lipoxygenase Enzymes

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Metal-mediated abstractions of hydrogen atoms from organic substrates are important in a range of biochemical and industrial processes. Iron(III) centers, while not usually viewed as strong oxidants, are involved in the catalytic oxidation of ethylbenzene to styrene[1] and as the reactive site in lipoxygenase enzymes. Lipoxygenases are nonheme iron enzymes that catalyze the peroxidation of polyunsaturated fatty acids by molecular oxygen.[2] The accepted mechanism[3−5] for lipoxygenase action involves rate-determining hydrogen transfer from the 1,4-*Z,Z*-pentadiene-containing fatty acid to the iron(III) hydroxide active site. Reported here are reactions of an iron(III) complex [Fe(Hbim)-(H$_2$bim)$_2$](ClO$_4$)$_2$ (**2**) with 1,4-pentadiene substrates, including the first anaerobic oxidation of a fatty acid ester by an iron complex.[6] This work, and the reactions of another functional lipoxygenase model recently reported by Jonas and Stack,[7] support our proposal that the ability of metal-containing active sites to abstract hydrogen atoms is related to their thermodynamic affinity for H•.[8]

[Fe^{II}(H$_2$bim)$_3$](ClO$_4$)$_2$ (**1**) is synthesized from Fe(ClO$_4$)$_2$ and 2,2′-bi-imidazoline[9] (H$_2$bim). **Caution!** *Perchlorate salts may be explosive.* As reported, **1** reacts with molecular oxygen resulting in oxidation of the iron and deprotonation of one of the ligands forming [Fe^{III}(Hbim)(H$_2$bim)$_2$](ClO$_4$)$_2$ (**2**).[9] Complex **2** reacts with excess 9,10-dihydroanthracene (DHA) to form anthracene (90 ± 5%) and **1** (>98%) as monitored by optical and NMR spectroscopies (eq 1). Reactions in acetonitrile under nitrogen showed clean second-order kinetics for the conversion of **2** to **1** by UV−vis spectrophotometry (see Supporting Information). At 70 °C, the second-order rate constant for the disappearance of **2** (eq 1) is (3.1 ± 0.2) × 10^{-3} M^{-1} s^{-1}.[10] Eyring analysis of the rate constants (25−70 °C) gives $\Delta H^{\ddagger}$ = 11.6 ± 0.5 kcal/mol and $\Delta S^{\ddagger}$ = −36 ± 5 e.u. Reaction of 9,9,10,10-*d*$_4$-DHA is 4.0 ± 0.4 times slower than the protio derivative at 50 °C. This is comparable to the isotope effect observed for 1,4-cyclohexadiene oxidation by
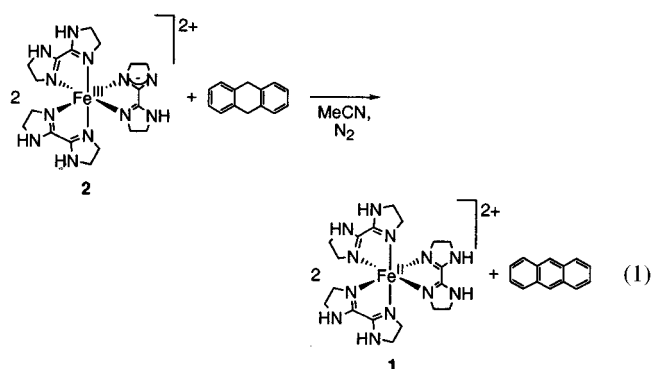
Jonas and Stack[7] but smaller than the very large values reported for the enzymes.[3]
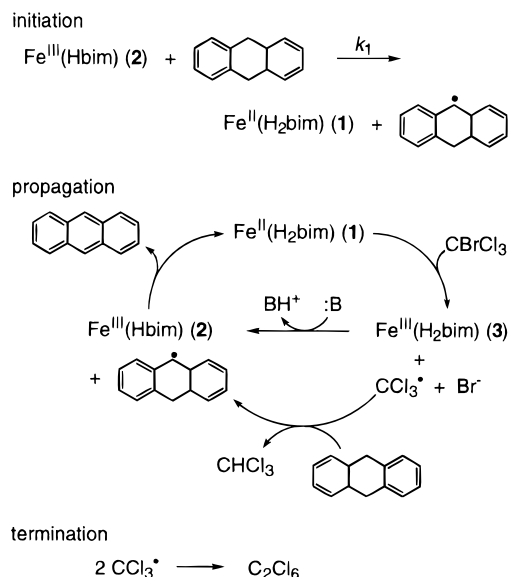
Complex **2** is a competent oxidant of hydrocarbons having C−H bond strengths ≤80 kcal/mol: DHA (78), xanthene (75.5), 1,4-cyclohexadiene (CHD, 77), and the ethyl ester of linoleic acid (∼78).[11] Oxidation of xanthene ((3.1 ± 0.1) × 10^{-3} M^{-1} s^{-1} at 70 °C) gives stoichiometric bixanthyl, the coupling product of the xanthyl radical. CHD is oxidized at (1.5 ± 0.1) × 10^{-3} M^{-1} s^{-1} at 70 °C forming benzene in 90 ± 10% yield by NMR. Oxidation of linoleic acid ethyl ester is slowest ((1.6 ± 0.1) × 10^{-4} M^{-1} s^{-1} at 70 °C). GC/MS analysis shows oxidation products two Daltons lighter than the fatty acid ester, most likely the 9,11,13- and 8,10,12-octadecatrienoic acid ethyl esters. These trienoic acids are metabolites of linoleic acid in certain plants, resulting from dehydrogenase rather than oxygenase activity.[12]

The oxidation of DHA is dramatically faster in oxygen-saturated acetonitrile. After 1 h at 70 °C, anthracene and anthraquinone are formed in a 0.42:1 ratio, and the yields are more than 100 times larger than expected based on the number of oxidizing equivalents of Fe(III) present. Under the same conditions, autoxidation of DHA in the absence of iron gives one-third as much anthracene and anthraquinone, in a ∼1:1 ratio. These observations are consistent with **2** acting as an initiator and modulator of autoxidation via hydrogen abstraction.

Reactions in the presence of CBrCl$_3$ were explored with the goal of trapping an intermediate hydrocarbon radical. Though no reaction is observed between **2** and CBrCl$_3$, the oxidation of DHA is accelerated by the presence of CBrCl$_3$. The reaction produces stoichiometric amounts of anthracene and the ferric complex [Fe^{III}(H$_2$bim)$_3$](ClO$_4$)$_3$ (**3**),[9a] as well as chloroform and hexachloroethane. Kinetic studies show a biphasic reaction, with the initial phase occurring at the same rate as **2** + DHA. After ∼10%
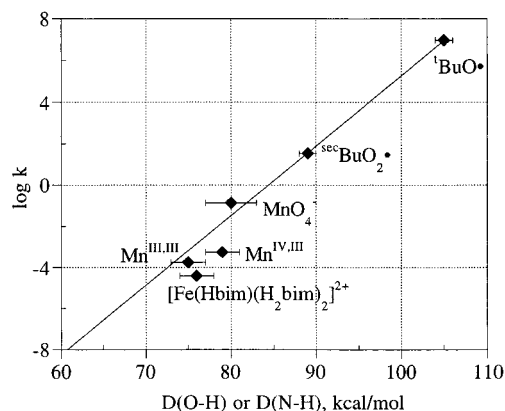
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(10) Errors are reported as one standard deviation about the mean.

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Scheme 1. Proposed Mechanism for the Iron-Mediated Oxidation of DHA by $CBrCl_3$

Figure 1. Log k (per reactive hydrogen) for hydrogen atom abstraction from DHA at 30 °C vs the strength of the O−H or N−H bond formed by the oxidant. $Mn^{IV,III}$ and $Mn^{III,III}$ represent $[Mn^{IV}Mn^{III}(\mu\text{-}O)_2(phen)_4]^{3+}$ and $[Mn^{III}_2(\mu\text{-}O)(\mu\text{-}OH)(phen)_4]^{3+}$, respectively. The line is an extrapolation from the oxygen radical rates. Data are from ref 8.

conversion of **2** to **1** (at 20 mM $CBrCl_3$), the second phase begins and the reaction accelerates. This is due to the rapid oxidation of **1** by $CBrCl_3$ to give **3** and $CCl_3^•$ (forming hexachloroethane in the absence of DHA). Consistent with this view, the rate of the initial phase does not depend on the concentration of $CBrCl_3$ but its duration does. The $CCl_3^•$ radical then abstracts a hydrogen atom from DHA; with d_{12}-DHA, $CDCl_3$ is formed. The 9-hydroanthracenyl radical is oxidized to anthracene by **2**, generating more **1**. No traces of bromoanthracenes are detected, due to the reactivity of such species with **1**.[13] The oxidation of DHA by **2**/$CBrCl_3$ is thus a metal-mediated radical chain mechanism (Scheme 1), initiated by the reaction of **2** with DHA. In the presence of a base strong enough to deprotonate **3** to **2**, the reaction becomes catalytic in iron as shown in the scheme.

The conversion of **2** to **1** on oxidation of substrates is a net gain of a hydrogen atom. The magnitude of the enthalpy for H$^•$ addition to **2**—the N−H bond strength in **1**—can be calculated from redox potential and pK_a data using a thermochemical cycle.[8,11b,14] Spectrophotometric titration of **3** with *N*-methylmorpholine in acetonitrile cleanly gives **2** and shows a pK_a of 17.5 ± 0.5. Cyclic voltammetry (acetonitrile, 0.1 M ^{n}Bu$_4$NPF$_6$), shows a reversible wave for the **1/3** couple at −0.31 ± 0.05 V vs Cp$_2$Fe$^{+/0}$. The derived N−H bond strength of 76 ± 2 kcal/mol is consistent with the ability of **2** to abstract H$^•$ from substrates with weak C−H bonds. Alternative mechanisms involving initial electron transfer are not possible because outer-sphere oxidation of DHA ($E° \cong 1.3$ V vs FeCp$_2^{+/0}$) by **2** is ca. 1.9 V uphill ($\Delta G° = 44$ kcal/mol),[15] much higher than the observed $\Delta G^‡$ of 22 kcal/mol at 298 K. Initial protonation transfer to **2** seems unlikely considering the low Brønsted and kinetic acidities of the hydrocarbon substrates, but is not precluded by the thermodynamics.

Rate constants for hydrogen atom transfer to main-group radicals typically correlate with the exo- or endothermicity of the H-atom transfer step.[16] A plot of this correlation (Figure 1) shows that the reactivity of **2** is close to what would be expected for an oxygen radical making a 76 kcal/mol bond to H$^•$. The rate constants for H-atom abstraction from DHA by permanganate, $[Mn^{IV}Mn^{III}(\mu\text{-}O)_2(phen)_4]^{3+}$, and $[Mn^{III}_2(\mu\text{-}O)(\mu\text{-}OH)(phen)_4]^{3+}$ also fit the single correlation within a couple of orders of magnitude.[8] It is remarkable that **2** is similar to the other oxidants because hydrogen is transferred to a nitrogen atom rather than an oxygen[17] and because the basic site is three bonds removed the redox active iron center. The relative rates per hydrogen—xanthene > DHA > CHD > linoleic acid—are reasonable based on the strengths of the C−H bonds being cleaved. Jonas and Stack's oxidation of DHA by $[Fe^{III}(PY5)(OMe)]^{2+}$ is quite a bit slower than predicted, as this oxidant forms an 8 kcal/mol stronger bond than **2** yet oxidizes DHA with essentially the same rate constant.[7] The origin of these differences are not yet apparent.

In conclusion, $[Fe(Hbim)(H_2bim)_2](ClO_4)_2$ (**2**) abstracts H from hydrocarbons with weak C−H bonds. DHA oxidation by **2** is within a factor of 50 of that expected for an oxygen radical that makes a 76 kcal/mol bond to H$^•$, despite the fact that the hydrogen is accepted by a nitrogen atom three bonds removed from the iron. This study supports our proposal that thermodynamic driving force is a primary determinant of hydrogen transfer reactivity and sheds new light on the mechanism of C−H bond oxidation by lipoxygenase and related desaturase enzymes.[12,18]

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