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The Iron(II)/Reductant (DH_2)-Induced Activation of Dioxygen for the Demethylation of *N*-Methylanilines: Reaction Mimic for the Cytochrome P-450/Reductase System

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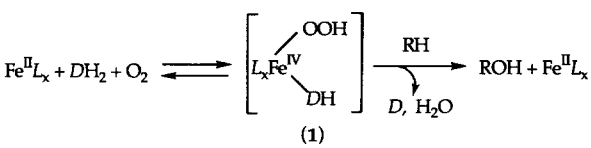
Abstract—Several iron(II) complexes [$Fe^{II}L_x$; $Fe^{II}(DPAH)_2$ ($DPAH_2$ = 2,6-dicarboxyl pyridine), $Fe^{II}(PA)_2$ (PAH = picolinic acid) and $Fe^{II}(bpy)_2^{2+}$ (bpy = 2,2′-bipyridine)] in combination with a reductant [DH_2; PhNHNHPh (mimic of dihydroflavin)] catalytically activate O_2 (1 atm) for the demethylation of *N*-methylanilines [$PhN(CH_3)_2$, $PhNH(CH_3)$]. This chemistry, which appears to involve a Fenton-like intermediate [$L_xFe^{IV}OOH(DH)$ (**1c**)], mimics that of the cytochrome P-450 monooxygenase/reductase proteins. Copyright © 1996 Elsevier Science Ltd

Introduction

The cytochrome P-450 monoxygenase proteins in combination with dihydroflavins catalytically activate dioxygen for the *N*-dealkylation of xenobiotic and naturally occurring amines.[1,2] Previous work has shown that in dry acetonitrile, anhydrous $Fe^{III}Cl_3$ activates HOOH to demethylate *N,N*-dimethylaniline,[3,4] which mimics the biological system. Several transition metal complexes [$Fe^{II}L_x$; $Fe^{II}(DPAH)_2$ ($DPAH_2$ = 2,6-dicarboxyl pyridine), $Fe^{II}(PA)_2$ (PAH = picolinic acid) and $Fe^{II}(bpy)_2^{2+}$ (bpy = 2,2′-bipyridine)] in combination with a reductant [DH_2; PhNHNHPh (mimic of dihydroflavins)] activate dioxygen for (a) the hydroxylation, ketonization and peroxidation of saturated hydrocarbons (e.g., cyclohexane), olefins (e.g., cyclohexene) and double allylic carbon centers (e.g., 1,4-cyclohexadiene)[5] and (b) the hydroxylation of phenol and substituted phenols.[6] Several of these processes parallel those of cytochrome P-450 proteins.[1]

The hydroxylation of hydrocarbons by iron(II) complexes [$Fe^{II}L_x$] in combination with O_2 and a reductant [DH_2] appears to involve a reactive intermediate (**1**) that has the energetics to break the C—H bond of an alkane (e.g., $\Delta H_{DBE} = 95.5$ kcal mol^{-1} for *c*-C_6H_{12}).[5]



(1)

Since the chemistry parallels that of the *cytochrome P-450 mono-oxygenase/reductase* proteins, the present study has been undertaken (a) to ascertain whether the $Fe^{II}L_x/DH_2/O_2$ systems demethylate *N*-methylanilines, (b) to characterize the reactive intermediates and (c) to ascertain the relevance of these systems as mechanistic mimics for the biological system.

Results

Table 1 summarizes the product profiles for three iron(II) [$Fe^{II}(DPAH)_2$, $Fe^{II}(PA)_2$ and $Fe^{II}(bpy)_2^{2+}$]/reductant [PhNHNHPh]/O_2 combinations in a 3:1 mol ratio of acetonitrile/pyridine [$(MeCN)_3py$] solvent with *N,N*-dimethylaniline (1 M) as the substrate. The system produces *N*-methylaniline (PhNHMe), *N*-methylformanilide [$PhN(CH_3)CH(O)$], the ring-hydroxylated product [dimethylaminophenol $HOC_6H_4NMe_2$] and *N*-methyl(*N*-methanol)aniline [$PhN(CH_3)CH_2OH$]. The 5 mM $Fe^{II}(DPAH)_2$/100 mM PhNHNHPh/1 M $PhNMe_2$ combination produces the largest yield [21 mM $PhNHCH_3$, 13 mM $PhNH(CH_3)CH(O)$, 8.2 mM $HOPhN(CH_3)_2$ and 7.4 mM $PhN(CH_3)CH_2OH$].

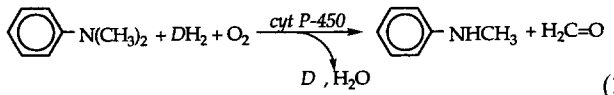
With 1 M PhNHMe the 5 mM $Fe^{II}(DPAH)_2$/50 mM PhNHNHPh/O_2 combination in 3:1 MeCN/py produces 7.9 mM $PhNH_2$, 3.1 mM PhNHCH(O) and 3.3 mM $PhN(CH_3)CH(O)$. The 5 mM $Fe^{II}(bpy)_2^{2+}$/50 mM PhNHNHPh/O_2 combination in MeCN produces similar yields with 9.4 mM $PhNH_2$, no PhNHCH(O) and 0.4 mM $PhN(CH_3)CH(O)$. With 1 M $PhNH_2$ the 5 mM $Fe^{II}(DPAH)_2$/50 mM PhNHNHPh/O_2 combination in 3:1 MeCN/py produces 6 mM *o*-$HOPhNH_2$ and 1.2 mM *p*-$HOPhNH_2$ as the only detectable products.

Discussion and Conclusion

The efficiency and selectivity for the demethylation process is superior with the $Fe^{II}(DPAH)_2$ complex

(Table 1). With a constant reductant concentration, the reaction efficiency (based on amount of reductant) increases the extent of demethylation and overall substrate oxidation as the catalyst concentration increases. At a concentration ratio of 10 mM PhNHNHPh/5 mM $Fe^{II}(DPAH)_2$ the efficiency for demethylation reaches a plateau, but the selectivity improves as the ratio is increased to 100 mM PhNHNHPh/5 mM $Fe^{II}(DPAH)_2$. Neither $Fe^{II}(PA)_2$ nor $Fe^{II}(bpy)_2^{2+}$ exhibit the efficiency or selectivity of $Fe^{II}(DPAH)_2$, but they both produce analogous product profiles [their optimal combinations are 3 mM $Fe^{II}(PA)_2$/50 mM DH_2 and 10 mM $Fe^{II}(bpy)_2^{2+}$/10 mM DH_2 in MeCN].

The results in Table 1 for the demethylation of *N,N*-dimethylaniline confirm that the various $Fe^{II}L_x/DH_2/O_2$ systems mimic the catalytic chemistry for cytochrome P-450 monooxygenase/reductase proteins:



(2)

However, in the chemical systems, the reductant (DH_2) is a competitive substrate, especially when present at the levels necessary to obtain adequate product for accurate assays. This may account for the observation that at higher concentrations nearly 40% of the reductant is consumed as a substrate.

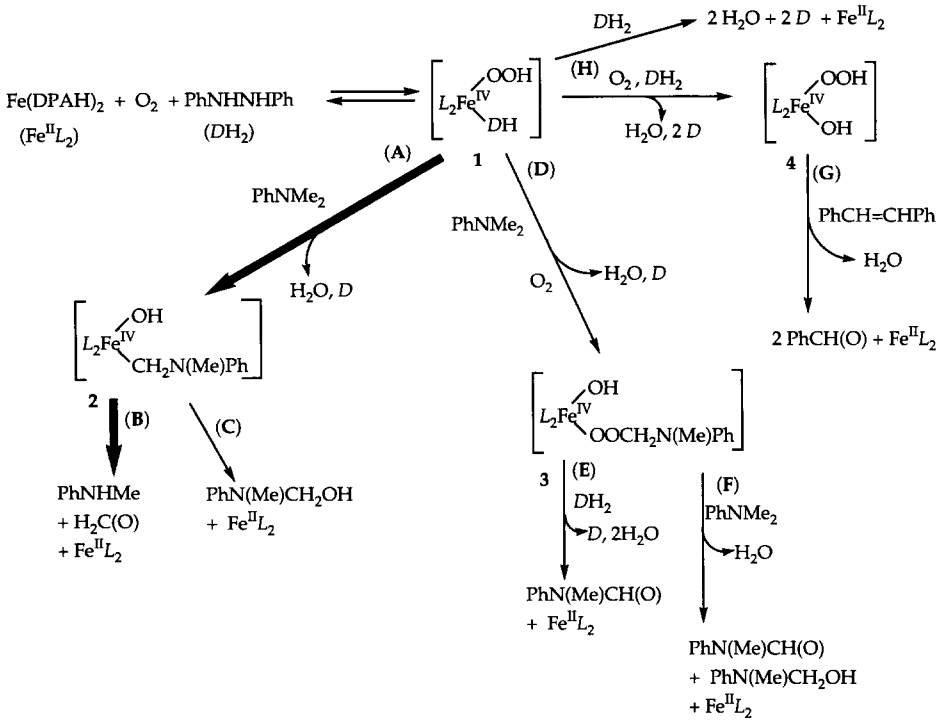
Scheme 1 outlines a set of proposed reaction paths that are in accord with the product profiles for $PhNMe_2$. Reduced iron complexes [e.g., $Fe^{II}(DPAH)_2$] reversibly bind dioxygen and in the presence of reductants (DH_2) appear to undergo H-atom addition to give reactive intermediate **1**. This hydroxylation intermediate has a reactivity and produces products that are similar to those for the reactive intermediates of Fenton reagents.[7,8]

In the case of *N,N*-dimethylaniline, we suggest that intermediate **1** (a) collapses to release *D* and an HO• group that abstracts an H atom from $PhN(CH_3)_2$ to form a water molecule and (b) binds the $PhN(CH_3)CH_2$• to the iron center in a concerted bond-breaking/bond-forming process to give inter-

Table 1. The $Fe^{II}L_x/DH_2$-induced activation of O_2 for the demethylation of *N,N*-dimethylaniline

$Fe^{II}L_x/DH_2$ ratio (mM/mM)	Products (mM, ±5%)[a]				rctn effcn (%)[b]	
	$PhNHCH_3$	RCH(O)[c]	$HOPhN(CH_3)_2$	RCH_2OH[c]	$PhNHCH_3$	Total
$Fe^{II}(DPAH)_2$						
1/10	0.5	2.0	0.5	0.4	5	54
1/30	2.5	3.9	1.4	1.3	8	53
1/50	4.3	5.5	2.5	2.1	9	46
3/50	8.6	8.0	4.9	3.2	17	65
5/10	1.9	2.6	0.8	0.4	19	83
5/30	5.8	5.2	2.8	1.5	19	68
5/50	9.6	7.6	4.1	2.8	19	63
5/100	21	13	8.2	7.4	21	61
$Fe^{II}(PA)_2$						
3/50	4.0	7.4	2.5	1.8	8	46
5/10	0.2	1.9	0.2	0.2	3	44
5/30	1.4	3.5	0.7	0.5	5	32
5/50	3.6	6.5	2.0	1.6	7	40
5/100	6.5	11	3.5	3.0	7	34
10/10	0	1.8	0.1	0.1	0	38
10/30	1.3	3.4	0.4	0.7	4	31
10/50	3.2	5.7	2.7	1.5	6	38
$Fe^{II}(bpy)_2^{2+}$[d]						
3/50	0.7	0.8	0.2	0.2	1	5
5/10	1.1	1.5	0	0	11	41
5/30	1.5	1.5	0.3	0.1	5	16
5/50	1.9	1.6	0.3	0.2	4	11
10/10	2.0	1.4	0.1	0	20	49
10/30	2.6	1.5	0.2	0.1	9	20
10/50	2.3	1.7	0.2	0.2	5	12
10/10[e]	1.3	1.5	0.1	0	13	29
10/30[e]	2.5	2.0	0.4	0	9	16
10/50[e]	4.2	3.0	1.2	0	9	17

[a] *N,N*-Dimethylaniline (1 M), $Fe^{II}L_x$ and DH_2 (PhNHNHPh) combined in $(MeCN)_3py$ to give indicated initial concentrations in a total volume of 5.0 mL. Product solutions were analysed by capillary-column gas chromatography and GC–MS after a reaction time of 3 h at 24 ± 2 °C.
[b] Per cent reaction efficiency, mmol of $PhNHCH_3$ per mmol of DH_2; total per cent reaction efficiency, mmol of $PhNHCH_3$, RCH(O) (2×), $HOPhNH(CH_3)_2$ and RCH_2OH per mmol of PhNHNHPh.
[c] R = $PhN(CH_3)$—.
[d] Solvent system was MeCN.
[e] Solvent system was $MeCN_3py$.

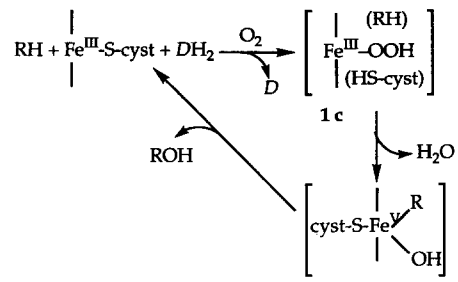


Scheme 1. $Fe^{II}L_x/DH_2$-induced activation of O_2 for reaction with *N*-methylanilines and *cis*-stilbene.

mediate **2** (path **A**). Transfer of the Fe-bound $PhN(Me)CH_2\bullet$ group to yield either (a) *N*-methylaniline, formaldehyde and $Fe^{II}(DPAH)_2$ (path **B**) or (b) $PhN(Me)CH_2OH$ and $Fe^{II}(DPAH)_2$ (path **C**). Intermediate **1** also can react as in path **A**, except the radical couples with O_2 to give $PhN(CH_3)CH_2OO\bullet$, which couples to the iron center to give intermediate **3** (path **D**). The latter either (a) reacts with another DH_2 to produce PhN(Me)CH(O) and $Fe^{II}(DPAH)_2$ (path **E**) or (b) reacts with a second $PhN(CH_3)_2$ to produce PhN(Me)CH(O), $PhN(Me)CH_2OH$ and FeII(DPAH)$_2$ (path **F**).

The pathways outlined in Scheme 1 involve H-atom transfer by iron/reductant-activated dioxygen intermediates. This is in contrast to earlier proposals that the reactive intermediate of cyctochrome P-450 proteins involves an $Fe^V=O$ center that facilitates O-atom transfer to olefins [e.g., *cis*-stilbene (PhCH=CHPh)] to produce their epoxides.[9,10] The present systems dioxygenate *cis*-PhCH=CHPh via intermediate **4** and path **G** of Scheme 1 and transform cyclohexene (c-C_6H_{12}) (via species **1** and paths **D**, **E** and **D**, **F**) into c-$C_6H_{10}(O)$ and c-$C_6H_{11}OH$, respectively.[5] Such an absence of olefin epoxidation is consistent with the observations for cytochrome P-450 monoxygenase/reductase proteins.[11]

Futhermore, the present systems hydroxylate aromatic substrates via species **1**,[6] which is in accord with the biological system. Hence, we suggest that the latter activates O_2 via H-atom transfer through an intermediate similar to **1** that reacts via the pathways of Scheme 1 [equation (3)].



(3)

We believe this is more reasonable chemistry for a biological matrix with a dihydroflavin reductase than the high-energy pathways of electron transfer or O-atom transfer.

Experimental

Equipment

The reaction products were separated and identified with a Hewlett Packard 5880A Series gas chromatograph equipped with a HP-1 capillary column (cross-linked methyl silicone gum phase, 12 m × 0.2 mm i.d.) and by GC–MS (Hewlett Packard 5790A Series gas chromatograph with a mass-selective detector).

Chemicals and reagents

The reagents for the investigations and syntheses were the highest purity commercially available and were used without further purification. Burdick and Jackson 'distilled in glass' grade acetonitrile (MeCN, 0.004%

H_2O) and pyridine (py, 0.014% H_2O) were used as solvents. All compounds were dried in vacuo over $CaSO_4$ for 24 h prior to use. Picolinic acid (PAH, 99%), 2,6-pyridine-dicarboxylic acid ($DPAH_2$, 99%), 2,2′-bipyridine (bpy, 99+%) and 1,2-diphenylhydrazine (95%) were obtained from Aldrich. The organic substrates obtained from Aldrich included: *N,N*-dimethylaniline (99%), *N*-methylaniline (99%) and aniline (99%).

Syntheses of $(Me_4N)PA$ and $(Me_4N)DPAH$

Tetramethylammonium picolinate [$(Me_4N)PA$] and tetramethylammonium dipicolinate [$(Me_4N)DPAH$] salts were prepared by the neutralization of picolinic acid (PAH) and 2,6-pyridine-dicarboxylic acid ($DPAH_2$) with tetramethylammonium hydroxide pentahydrate in aqueous aqueous solution. $(Me_4N)PA$ was recrystallized from acetonitrile and $(Me_4N)DPAH$ from 95% MeCN/5% MeOH. The hydroscopic products were stored under vacuum.

$[Fe^{II}(MeCN)_4](ClO_4)_2$

The $[Fe^{II}(MeCN)_4](ClO_4)_2$ complex was prepared by multiple recrystallizations of $[Fe^{II}(H_2O)_6](ClO_4)_2$ from MeCN.

Iron(II) bis(picolinate) and iron(II) bis(dipicolinate) solutions

Solutions of $Fe^{II}(PA)_2$ and $Fe^{II}(DPAH)_2$ were prepared in situ by mixing $[Fe^{II}(MeCN)_4](ClO_4)_2$ with stoichiometric ratios of ligand anion.

Iron(II) bis(2,2′-bipyridine) solutions

Solutions of $Fe^{II}(bpy)_2^{2+}$ were prepared in situ by mixing $[Fe^{II}(MeCN)_4](ClO_4)_2$ in MeCN with stoichiometric ratios of bpy.

Methods

The investigations of O_2/reductant (DH_2) activation by the iron complexes ($Fe^{II}L_x$) used solutions that contained 1.0 M substrate (RH), 1–10 mM $Fe^{II}L_x$, and 5–100 mM DH_2 in 3–4 mL of the appropriate solvent. The total solution volumes were 5.0 mL. The process was initiated by the addition of 1 atm of O_2 (7 mM) into the septum-covered glass reaction cell (volume, 21 mL; 16 mL of headspace). After 3 h with constant stirring at room temperature (24±2 °C), samples of the reaction solutions were injected into a capillary-column gas chromatograph for analysis. In some cases, the reaction was quenched with water and the product solution was extracted with diethyl ether. Product species were characterized by GC–MS. Reference samples were used to confirm product identifications and to produce standard curves for quantitative assays of the product species.

The experiments were designed to be limited by reductant (DH_2) in order to (a) evaluate the primary reaction efficiency with respect to DH_2, (b) minimize secondary reactions with the primary products and (c) limit the extent that DH_2 acted as a competitive substrate.

Acknowledgments

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