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Iron(II)/Reductant(DH_2)-Induced Activation of Dioxygen for the Hydroxylation and Ketonization of Hydrocarbons; Mimics for the Cytochrome P-450 Hydroxylase/Reductase System

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Abstract—Several metal complexes {$Fe^{II}(DPAH)_2$ ($DPAH_2$ = 2,6-dicarboxyl pyridine), $Fe^{II}(PA)_2$ (PAH = picolinic acid), $Fe^{II}(bpy)_2^{2+}$, $Fe^{II}(OPPh_3)_4^{2+}$, $(Cl_8TPP)Fe^{III}X$ (X=Cl, OH, SCH_2Ph) [Cl_8TPP = tetrakis (2,6-dichlorophenyl)porphyrin], (TPP) $Fe^{III}Cl$ (TPP = tetraphenylporphyrin), and $Cu^{I}(tpy)_2^{+}$ (tpy = 2,2'-6,2"-terpyridine)} in combination with one of several reductants [DH_2; PhNHNHPh (mimic of dihydroflavin), $PhNHNH_2$, ascorbic acid (H_2asc), and $PhCH_2SH$ (model ligand for cysteine residue)] catalytically activate O_2 (1 atm) for the hydroxylation of saturated hydrocarbons (e.g. c-C_6H_{12} → c-$C_6H_{11}OH$). This chemistry closely parallels that of cytochrome P-450 proteins, and both appear to involve a Fenton-like reactive intermediate), [$L_xFeOOH(DH)$]. With cyclohexene as the substrate the dominant product is its ketone (as well as significant amounts of its hydroperoxide). 1,4-Cyclohexadiene (with two double-allylic carbon centers) undergoes dehydrogenation to give benzene, but also yields substantial amounts of phenol via ketonization of an allylic carbon. The 1:1 $Fe^{II}(bpy)_2^{2+}$/($PhNHNH_2$ or H_2asc), $Fe^{II}(PA)_2/H_2asc$, and $(Cl_8TPP)Fe^{III}Cl/PhNHNH_2$ combinations initiate the autoxidation of 1,4-cyclohexadiene with turnover numbers (moles of product per mole of reductant) from 71 to 26, respectively (α-tocophenol reduces the turnover numbers by 20–80 %). With respect to aerobic biology, the present results indicate that dysfunctional transition metals (degradation products of metalloproteins) in combination with biological reductants activate O_2 for reaction with organic substrates. The level of activation is similar to that for Fenton reagents and cytochrome P-450 hydroxylases. Hence, dysfunctional transition metals, reductants, and O_2 are a hazardous combination within a biological matrix.

Introduction

Some years ago we reported that $Fe^{II}(DPAH)_2$ ($DPAH_2$ = 2,6-dicarboxyl pyridine) in 3:1 MeCN:pyridine [$(MeCN)_3py$] catalytically activates O_2/PhNHNHPh combinations to hydroxylate saturated hydrocarbons.[1] Because the reactivity pattern of the system closely parallels that of the *methane monooxygenase*[2,3] and *cytochrome P-450 hydroxylases*,[4] a similar reactive intermediate for the hydroxylation of saturated hydrocarbons is likely.

Fenton reagents (1:1 $Fe^{II}L_x$/HOOH) also hydroxylate saturated hydrocarbons via a proposed reactive intermediate (**1**) (a nucleophilic adduct)[5]

$$Fe^{II}L_x + HOOH \underset{}{\overset{B}{\rightleftharpoons}} \underset{\mathbf{1}}{[L_x{}^{-}Fe^{II}OOH(BH^{+})]}. \quad (1)$$

Although there has been a long-held belief that Fenton reagents react via the production of free hydroxyl radicals (HO·), their product profiles and substrate reactivity parameters are different, and are dependent upon the transition metal, the ligand, and the solvent matrix (which is not the case for free HO·).

The Fenton reactive intermediate (**1**) has been proposed to react with hydrocarbon substrates (RH) as a stabilized HO· and anchors the resultant carbon radical via an iron carbon bond[5,6]

$$\mathbf{1} + RH \xrightarrow[H_2O]{} \left[L_xFe^{IV}\begin{matrix}OH\\R\end{matrix}\right] \longrightarrow ROH + Fe^{II}L_x. \quad (2)$$

In the presence of O_2 (1 atm) species **1** forms an adduct [**1**(O_2)] that preferentially ketonizes methylenic carbon centers (*oxygenated Fenton chemistry*) [e.g. cyclohexane (c-C_6H_{12})][6],

$$\mathbf{1}(O_2) + c\text{-}C_6H_{12} \xrightarrow[H_2O]{} \underset{\mathbf{6}}{\left[L_xFe\begin{matrix}OH\\OOC_6H_{11}\end{matrix}\right]} \xrightarrow[H_2O]{c\text{-}C_6H_{12}} c\text{-}C_6H_8(O) + c\text{-}C_6H_{11}OH. + Fe^{II}L_x \quad (3)$$

Although the $Fe^{II}(DPAH)_2$ complex in 2:1 pyridine: HOAc solvent autoxidizes via the production of HOOH [with subsequent formation of **1** and **1**(O_2) and reaction with hydrocarbon substrates],[7,8] in 3:1 MeCN:pyridine [$(MeCN)_3py$] autoxidation occurs without formation of HOOH [4 $Fe^{II}(DPAH)_2 + O_2 \rightarrow 4\ Fe^{III}(DPA)(DPAH) + 2\ H_2O$] and any reaction with hydrocarbon substrates.[1] Hence, in the latter solvent the $Fe^{II}(DPAH)_2$-induced activation of O_2/reductase (DH_2; PhNHNHPh) combinations for the hydroxylation of hydrocarbons appears to occur via a mechanism that is similar to those for *cytochrome P-450 hydroxylase* and *methane monooxygenase hydroxylase* proteins.

This proposition has prompted a systematic study of O_2 activation by several transition metal complexes {$Fe^{II}(DPAH)_2$ ($DPAH_2$ = 2,6-dicarboxylpyridine), $Fe^{II}(PA)_2$ (PAH = picolinic acid), $Fe^{II}(bpy)_2^{2+}$, $Fe^{II}(OPPh_3)_4^{2+}$, $(Cl_8TPP)Fe^{III}X$ (X=Cl, OH, SCH_2Ph) [Cl_8TPP = tetrakis (2,6-dichlorophenyl)porphyrin], (TPP)$Fe^{III}Cl$ (TPP = tetraphenylporphyrin), and $Cu^{I}(tpy)_2^{+}$

(tpy = 2,2'-6-2"-terpyridine)} in combination with one of several reductants [DH_2; PhNHNHPh (mimic of dihydroflavin), $PhNHNH_2$, ascorbic acid (H_2asc), and $PhCH_2SH$ (1/2 DH_2; model ligand for cysteine residue)] for the hydroxylation, ketonization, and peroxidation of saturated hydrocarbons [e.g. cyclohexane (c-C_6H_{12})], olefins [e.g. cyclohexene (c-C_6H_{10})], and double-allylic carbon centers [e.g. 1,4-cyclohexadiene (c-C_6H_8)]. The goals have been (a) to characterize the reactive intermediates for the hydroxylation, ketonization, and peroxidation of hydrocarbon substrates, (b) to correlate these and their reactivities with those for the related Fenton (**1**, equations 1 and 2) and oxygenated Fenton [**1**(O_2), equation 3] reagents, and (c) to ascertain the relevance of these systems as mechanistic models for the cytochrome P-450 and methane monooxygenase hydroxylases.

During the past decade evidence has mounted that the combination of dysfunctional iron (or copper), ambient dioxygen (O_2), and biological reductants (NADH/ flavoproteins, thiols, amines, and ascorbic acid) is the fundamental cause of aging and induces several disease states (eschemia/reperfusion injury, heart disease, cancer, and neurological disorders).[9–13] (Dysfunctional metals in biology result, in part, from the degradation of metalloproteins.) Most believe that such combinations yield free oxy radicals (HO·, RO·, HO·/O_2^{-}·, and ROO·) via various redox cycles. However, Nature's most potent functional oxidants do not involve oxy-radical reactive intermediates {e.g. compound I of *horseradish peroxidase* reacts via [(por^{+}·)Fe^{IV}=O]; and cytochrome P-450 hydroxylates saturated hydrocarbons and aromatic rings, probably via [(por)(RS)Fe^{IV}OOH, DH]}.[14] Because the present study involves the equivalent of dysfunctional iron, reductants, and O_2 in biological systems, the results may provide insight to the mechanisms for oxygen toxicity.

Results

The product yields for three model substrates [cyclohexane (c-C_6H_{12}), cyclohexene (c-C_6H_{10}), and 1,4-cyclohexadiene (c-C_6H_8, 1,4-CHD)] that result from their combination with iron and copper complexes and various reductants [DH_2; PhNHNHPh (mimic of dihydroflavin), $PhNHNH_2$, ascorbic acid (H_2asc), and $PhCH_2SH$ (DH_2/2; mimic for a cysteine residue)] are summarized in Table 1. The dominant product for saturated hydrocarbons (c-C_6H_{12}) with iron catalysts and hydrazine reductants (PhNHNHPh and $PhNHNH_2$) is the alcohol (c-$C_6H_{11}OH$). All of the systems (except $PhCH_2SH$) produce some alcohol (kinetic isotope effects, $[K] = k_{c\text{-}C_6H_{12}}/k_{c\text{-}C_6D_{12}} = 1.0 \rightarrow 2.8$) as well as ketone [c-$C_6H_{10}$(O)] ($[K] = 4 \rightarrow 9$). With cyclohexene (c-$C_6H_{10}$) as the substrate, the dominant product is the ketone [c-C_6H_8(O)] plus substantial amounts of its hydroperoxide (c-C_6H_9OOH) for some systems. Only the iron-porphyrin catalysts produce significant amounts of alcohol from c-C_6H_{10}. As the ratio of reductants to substrate (DH_2/RH) is reduced from 1/20 to 1/200 the product yield per reductant increases and the dominant product for c-C_6H_{12} becomes its ketone (Table 2). The reaction efficiency approaches 100 % as the reductant: catalyst ratio approaches unity (the reductant is a competitive substrate).

Although 1,4-cyclohexadiene (c-C_6H_8; with two double-allylic carbon centers) readily undergoes dehydrogenation to give benzene (PhH), significant amounts of phenol (PhOH) are formed (apparently via the initial ketonization of an allylic carbon).[19] When the reductant (DH_2)/catalyst (FeL_x) ratio approaches unity, several systems initiate the autoxidation of c-C_6H_8 to form benzene (PhH) (with O_2/DH_2 turnovers of 50–70, Table 3). Other FeL_x/DH_2 combinations fail to exhibit any autoxidation. However, the combination of 1 mM $(Cl_8TPP)Fe^{III}OH$ and 50 mM $PhNHNH_2$ with O_2 and c-C_6H_8 in acetonitrile initiates an autoxidation cycle with more than three O_2/DH_2 turnovers (Table 1). The ability of ascorbic acid (H_2asc) in combination with $Fe^{II}(bpy)_2^{2+}$ or $Fe^{II}(PA)_2$ to initiate an autoxidation of double-allylic centers is surprising in view of its reputation as a antioxidant (Table 3). The addition of α-tocophenol to the active autoxidation-initiators of Table 3 reduces their turnover numbers by 25–80 %.

The thiol reductant ($PhCH_2SH$) is not effective with most catalysts for the oxygenation of saturated hydrocarbons (up to 95 % remains unreacted at the end of the experiment, Table 1). With cyclohexene (c-C_6H_{10}) as the substrate, $Fe^{II}(DPAH)_2$ becomes the most effective catalyst (the substrate induces $PhCH_2SH$ to be a reductant). However, the reactivity of the $Fe^{II}(DPAH)_2$/$PhCH_2SH$, O_2/c-C_6H_{10} system is highly dependent on the solvent matrix; an MeCN: pyridine mol-ratio of 3:1 [$(MeCN)_3py$] is optimum with 60 % of the $PhCH_2SH$ reactive (Table 4). The $Fe^{II}(OPPh_3)_4^{2+}$ complex is unique among the catalysts in its ability to induce the almost complete (99 %) autoxidation of $PhCH_2SH$ in the absence of a hydrocarbon substrate (Table 5). In contrast, $Fe^{II}(DPAH)_2$ induces only a 4 % transformation. However, in the presence of 1 M c-C_6H_{10} it induces an autoxidation of about 60 % of the $PhCH_2SH$ (Table 5). With 100 mM $PhCH_2SH$ its 60 % autoxidation is accompanied by the formation of oxygenated-substrate products (Table 4; oxidation of a 60-mM concentration of $PhCH_2SH$ yields a 28-mM concentration of substrate products).

Discussion and Conclusions

Hydroxylase mimics

The results in Table 1 for the hydroxylation of cyclohexane (c-C_6H_{12}) confirm that the various $ML_x/DH_2/O_2$ systems mimic the catalytic chemistry for the *cytochrome P-450 hydroxylase* (thiolated heme)[20] and *methane monooxygenase* proteins (equation 4).[2,3]

$$RH + O_2 + DH_2 \xrightarrow[\text{cyt P-450}]{\text{MMO or}} ROH + D + H_2O. \quad (4)$$

However, in the chemical systems the various reductants (DH_2) are competitive substrates, especially when

Table 1. $Fe^{II}L_x$/reductant (DH_2)-induced activation of O_2 for the oxygenation of hydrocarbons (RH) in $(MeCN)_3py$[a]

catalyst (3 mM)	products (mM, ±5%)[b] c-C_6H_{12} ROH [K][c]	c-C_6H_{12} R'(O) [K][c]	c-C_6H_{10} ROH	c-C_6H_{10} R'(O)	c-C_6H_{10} ROOH	c-C_6H_8 (1,4-CHD) PhH	c-C_6H_8 (1,4-CHD) PhOH
A. PhNHNHPh (50 mM)							
$Fe^{II}(DPAH)_2$	7.1 [1.6]	3.9 [6]	0	8.4	6	12	5.6
$Fe^{II}(PA)_2$	6.3 [1.7]	3.4 [7]	0	7.5	5	6.5	5.3
$Fe^{II}(bpy)_2^{2+}$	4.9 [1.9]	2.0	0	4.8	4	3.2	4.7
$Fe^{II}(OPPh_3)_4^{2+}$	3.5	1.8	0	4.7	3	8.8	4.3
$(Cl_8TPP)Fe^{III}Cl$(1 mM)/MeCN	10.2	0.8	4.3	1.0	3		
$(Cl_8TPP)Fe^{III}OH$(1 mM)/MeCN	7.8	1.7	3.7	1.8	4.5		
$(Cl_8TPP)Fe^{III}SCH_2Ph$(1 mM)/MeCN	8.4	1.3	3.1	1.1	3.0		
(TPP)$Fe^{III}Cl$(1 mM)/MeCN	6.5	<0.5	2.4	0.4	1.5		
$[Cu^I(tpy)_2^+]$	1.3 [2.0]	4.8 [4]	0	5.0	0	0	6.0
B. $PhNHNH_2$ (50 mM)							
$Fe^{II}(DPAH)_2$	6.7 [2.5]	2.7	0	16	0	24	0
$Fe^{II}(PA)_2$	6.7 [2.3]	4.5 [9]	0	18	0	56	0
$Fe^{II}(bpy)_2^{2+}$	1.7 [1.9]	2.1	0	14	0	33	0
$Fe^{II}(OPPh_3)_4^{2+}$	2.4	1.7	0	13	0	17	0
$(Cl_8TPP)Fe^{III}OH$ (1 mM)/MeCN						154	13
C. ascorbic acid (50 mM)							
$Fe^{II}(DPAH)_2$	1.3 [1.0]	3.1 [6]	0	6.0	3	19	0
$Fe^{II}(PA)_2$	2.4 [1.0]	5.7 [4]	0	7.4	3	28	3.7
$Fe^{II}(bpy)_2^{2+}$	1.5 [1.0]	1.5 [5]	0	4.0	0	30	3.6
$Fe^{II}(OPPh_3)_4^{2+}$	1.9 [1.1]	2.8 [4]	0	5.1	2	30	3.7
D. $PhCH_2SH$ (100 mM)							
$Fe^{II}(DPAH)_2$	0 (95)[d]	0	3.5(40)[d]	16	8	40 (83)[d]	4.0
$Fe^{II}(PA)_2$	1.8 [2.8] (4.8)	3.0 [6]	0 (57)	4.1	3	86 (2)	4.3
$Fe^{II}(bpy)_2^{2+}$	0 (40)	0	0 (39)	3.0	0	1.8 (38)	3.5
$Fe^{II}(OPPh_3)_4^{2+}$	0 (2)	0	0 (2)	3.2	0	23 (<1)	3.9
$(Cl_8TPP)Fe^{III}SCH_2Ph$ (1 mM/MeCN)[e]	5.2	1.8	4.8	2.8	8		

[a] To the combination of 3 mM catalyst (ML_x), 1 M substrate (RH), and O_2 (1 atm, 7 mM) in 3:1 MeCN: pyridine was added sufficient reductant (DH_2) to give an initial concentration of 50 mM DH_2 (100 mM for $PhCH_2SH$) PAH, picolinic acid; $DPAH_2$, 2,6-dicarboxyl-pyridine; bpy, 2,2'-bipyridine; Ph_3PO, triphenylphosphine oxide; tpy, 2,2'-6,2"-terpyridine.
[b] The product solutions were analyzed by capillary-column gas chromatography and GC–MS after a reaction time of 4 h at 24±2 °C.
[c] $[K] = [k_{c\text{-}C_6H_{12}}/k_{c\text{-}C_6H_{12}}]$, kinetic isotope effect.
[d] Concentration (mM) of $PhCH_2SH$ (unreacted) at end of experiment.
[e] Experiment used 25 mM $PhCH_2SH$. The actual product yields have been multiplied by four to give the tabulated data.

present at the levels necessary to obtain adequate product for accurate assays [the $Fe^{II}(DPAH)_2$/PhNHNHPh system approaches stoichiometric efficiency at low reductant concentrations, Table 2] (equation 5)

$$c\text{-}C_6H_{12} + O_2 + PhNHNHPh \xrightarrow{Fe^{II}(DPAH)_2} c\text{-}C_6H_{11}OH + PhN{=}NPh + H_2O. \quad (5)$$

Although the $Fe^{II}(DPAH)_2$ complex in 2:1 pyridine: acetic acid is autoxidized via the transient production of hydrogen peroxide and subsequent oxygenated Fenton chemistry,[6,8]

$$2\ Fe^{II}(DPAH)_2 + O_2 \xrightarrow{(py)_2HOAc} 2\ Fe^{III}(DPA)(DPAH) + HOOH \quad (6)$$

Table 2. Effect of reductant (DH_2): substrate (RH, 1 M) ratio on the $Fe^{II}(DPAH)_2$ (3 mM)/ reductant (DH_2)-induced activation of O_2 for the oxygenation of hydrocarbons in $(MeCN)_3py$.[a]

PhNHNHPh	products (mM, ±5%)					
	c-C_6H_{12}			c-C_6H_{10}		
(DH_2, mM)	ROH	R'(O)	effcny,%[b]	R'(O)	ROOH	effcny,%[b]
50	7.1	3.9	30	8.4	6	58
20	2.9	2.6	41	3.8	2	58
10	1.6	2.0	56	2.0	1	60
5	1.0	1.6	84	1.4	<1	86

[a]See Table 1 for experimental conditions and protocols.
[b]100 % represents one ROH per DH_2 and/or one R'(O) (or ROOH) per two DH_2.

Table 3. $Fe^{II}L_x$ (3 mM)/reductant (DH_2)/O_2 (1 atm)-initiated autoxidation of 1,4-Cyclohexadiene (1 M) in $(MeCN)_3py$[a]

	yield of PhH (mM, ±5%) [turnovers, product/DH_2]				
	$Fe^{II}(bpy)_2^{2+}$			$Fe^{II}(PA)_2$	
DH_2 (mM)	$PhNHNH_2$	PhNHNHPh	H_2asc	H_2asc	PhNHNHPh
50	38 [0.8]	3 [0.2]	30 [0.6]	28 [0.6]	7 [0.1]
20	29 [1.5]	7 [0.4]	102 [5.1]	144 [7.2]	0
10	282 [28]	5 [0.5]	184 [18]	252 [25]	0
5	355 [71][b,c]	0	269 [54][d]	321 [64][e,f,g]	0

[a]See Table 1 for experimental conditions and protocols.
[b]The presence of 10 mM α-tocopherol reduced the yield to 67 mM PhH.
[c]With 1 mM $(Cl_8TPP)Fe^{III}Cl$ as the catalyst in MeCN the yield was 130 mM PhH and 4 mM PhOH.
[d]The presence of 5 mM α-tocopherol reduced the yield to 130 mM PhH.
[e]The presence of 50 mM α-tocopherol reduced the yield to 242 mM PhH.
[f]With 5 mM $Fe^{II}(DPAH)_2$ as the catalyst the yield was 10 mM PhH.
[g]With 1 mM $(Cl_8TPP)Fe^{III}OH$ in MeCN the yield was 83 mM PhH and 1.3 mM PhOH.

in 3:1 MeCN:pyridine the autoxidation in the presence of c-C_6H_{12} does not yield any product from Fenton chemistry or oxygenated Fenton chemistry.[1]

For the hydroxylation of c-C_6H_{12} by the {[$Fe^{II}(DPAH)_2$ or $Fe^{II}(PA)_2$]/PhNHNHPh} systems (Table 1) the kinetic-isotope-effects ([K] = $k_{c\text{-}C_6H_{12}}/k_{c\text{-}C_6D_{12}}$) are 1.6 and 1.7, respectively, which are essentially the same as those for the $Fe^{II}(DPAH)_2$/HOOH (1.7) and $Fe^{II}(PA)_2$/HOOH (1.8) Fenton systems.[5] Although this infers a similar reactive intermediate, the Fenton reagents in the presence of pyridine transform c-C_6H_{12} to (c-C_6H_{11})py rather than the alcohol.[5,6] Also, in contrast to their hydroxylation analogues (Table 1), the $Fe^{II}(bpy)_2^{2+}$/HOOH, $Fe^{II}(OPPh_3)_4^{2+}$/HOOH, and $Cu^{I}(tpy)_2^{+}$/HOOH Fenton combinations are unreactive with c-C_6H_{12}.

The variation in the yield of c-$C_6H_{11}OH$ (and in [K]) with reductant (DH_2) for the $Fe^{II}(PA)_2/O_2$/c-C_6H_{12}/DH_2 system further confirms that its reactive intermediate is different from that for Fenton chemistry (produced by the nucleophilic addition of HOOH to an electrophilic ML_x center).[5] Also, the product ratio [c-$C_6H_{11}OH$/c-C_6H_{10}(O)] via iron-porphyrin-catalyzed hydroxylation is at least 4.6 (Table 1, [K] = 2.4 for c-$C_6H_{11}OH$ production) vs about 1 for a $(Cl_8TPP)Fe^{II}$(py)2/HOOH (100 mM) oxygenated Fenton system ([K] = 2.0 for c-$C_6H_{11}OH$ production).[6]

Table 4. The effect of solvent on the product profile for the $Fe^{II}(DPAH)_2$ (3 mM)/$PhCH_2SH$ (100 mM)/c-C_6H_{10} (1 M)/O_2 (1 atm) system.[a]

	substrate products (mM, ±5%)			reductase and its products (mM, ±5)				
solvent	c-C_6H_9OH	c-$C_6H_8(O)$	c-C_6H_9OOH	$PhCH_2SH$	$PhCH_2SSCH_2Ph$	PhCH(O)	PhC(O)OH	Σ^b
$(MeCN)_4py$		3.5	2	62	16	3		97
$(MeCN)_3py$	3.5	16	8	40	16	17	9	98
$(MeCN)_2py$		2.3	<1	81	5	2		93
(MeCN)py		2.3	<1	94		3		97

[a]See Table 1 for experimental conditions and protocols.
[b]Summation of reductant and its products (includes 2 x $PhCH_2SSCH_2Ph$ concentration).

Table 5. Iron(II)-induced autoxidation of thiols ($PhCH_2SH$) by O_2 (1 atm, 7 mM) in $(MeCN)_3py$[a]

		products (mM, ±5%)			
catalyst (3 mM)	$PhCH_2SH$(mM)	$PhCH_2SSCH_2Ph$	PhCH(O)	PhC(O)OH	%[b] conv
$Fe^{II}(OPPh_3)_4^{2+}$	100	47	5		99
$Fe^{II}(DPAH)_2$	100	1	2		4
$Fe^{II}(DPAH)_2$/c-C_6H_{10}(1 M)	100	16	17	9	58
$Fe^{II}(DPAH)_2$/c-C_6H_{10}(1 M)	50	11	9	3	68
$Fe^{II}(DPAH)_2$/c-C_6H_{10}(1 M)	20	3	5	1	60
$Fe^{II}(DPAH)_2$/c-C_6H_{10}(1 M)	10	1	3		50

[a]See Table 1 for experimental conditions and protocols.
[b]Summation of products (includes 2 × $PhCH_2SSCH_2$ concentration) divided by initial $PhCH_2SH$ concentration times 100; represents the percentage of $PhCH_2SH$ oxidized.

Ketonases

Although the 1:1 combination of $Fe^{II}(bpy)_2^{2+}$, $Fe^{II}(OPPh_3)_4^{2+}$, or $Cu^{I}(tpy)_2^{+}$ with HOOH does not result in an effective Fenton reagent, in the presence of O_2 these reagents [as well as $Fe^{II}(DPAH)_2$ and $Fe^{II}(PA)_2$] ketonize methylenic carbon centers (oxygenated Fenton chemistry via reactive-intermediate **5**)[5,6]

$$Fe^{II}(bpy)_2^{2+} + HOOH \underset{}{\overset{K<<1}{\rightleftharpoons}}$$

$$\underset{\mathbf{1}}{[(bpy)_2{}^{+}Fe^{II}OOH(BH^{+})]} \overset{O_2}{\rightleftharpoons} \underset{\mathbf{5}}{[\mathbf{1}(O_2)]}. \qquad (7)$$

The species **5** for $Fe^{II}(DPAH)_2$ and $Fe^{II}(PA)_2$ catalyze the ketonization of c-C_6H_{12} ([*K*], 2.4 and 2.5, respectively). All of the hydroxylation systems in Table 1 also ketonize methylenic-carbon centers [with c-C_6H_{12} its ketone is a secondary product for 50 mM PhNHNHPh, but the dominant product for 5 mM PhNHNHPh, Table 2; for $Fe^{II}(DPAH)_2$ and $Fe^{II}(PA)_2$ the respective (*K*) values are 6 and 7]. In contrast, with cyclohexene (c-C_6H_{10}) and the various non-porphyrin hydroxylation systems the dominant product is ketone [cyclohexene-3-one, c-$C_6H_8(O)$] and the secondary product is the hydroperoxide derivative [cyclohexene-3-hydroperoxide, c-C_6H_9OOH].

$$\text{c-}C_6H_{10} + 2\ O_2 + 2\ PhNHNHPh \xrightarrow{Fe^{II}(DPAH)_2}$$

$$\underset{(\text{c-}C_6H_9OOH)}{\text{c-}C_6H_8(O)} + 2\ PhN{=}NPh + \underset{(2\ H_2O)}{3\ H_2O}. \qquad (8)$$

Only the heme systems and the $Fe^{II}(DPAH)_2$/$PhCH_2SH$ combination yield measurable amounts of alcohol from c-C_6H_{10}.

Reactive intermediates

These considerations lead to three conclusions: (i) the ML_x/O_2, DH_2 systems do not initially produce HOOH

in their hydroxylation and ketonization of hydrocarbon substrates; (ii) these systems activate O_2 via a reductant (DH_2, with H-atom transfer) to produce an intermediate (**1d**) whose reactivity is dependent on the specific reductant. In contrast, Fenton catalysts activate HOOH via electrophile/nucleophile interactions to give **1** (equation 1). The hydroxylation intermediate (**1d**) has a reactivity and produces product profiles that are closely similar to that for the Fenton intermediate (**1**); a stabilized HO· (via a weak MO—OH bond). This bond energy in **1** is controlled by the Brønsted acidity of ML_x. By analogy a reasonable formation path and formulation of **1d** involves an initial reversible binding of O_2 followed by H-atom addition from DH_2

$$Fe^{II}(DPAH)_2 + O_2 \underset{}{\overset{(MeCN)_3py}{\rightleftharpoons}} [L_2Fe(O_2) \xrightarrow{DH_2} \underset{\mathbf{1d}}{[L_2Fe^{IV}(OOH)(DH)]} . \quad (9)$$

The FeO—OH bond energy in **1d** is dependent on the FeD—H bond energy, which in turn depends on the Fe—DH bond energy. On the basis of their electrochemical oxidation potentials, the N—H bond energies ($-\Delta G_{BF}$) for PhNHNHPh and $PhNHNH_2$ are estimated to be 76 and 74 kcal mol^{-1}, respectively.[21] This difference and lack of symmetry causes the reactivity of species **1d** to be reductant-dependent, especially with respect to the ketonization of cyclohexene (Table 1). With $Fe^{II}(DPAH)_2$ and $Fe^{II}(PA)_2$ the reactivities of their respective species **1** (Fenton) and species **1d** (hydroxylation) are essentially the same {[*K*] = 1.8, 1.7 (Fenton) vs [*K*] = 1.6, 1.7 (hydroxylation)}. In contrast, the species **1** for $Fe^{II}(bpy)_2^{2+}$/HOOH is unreactive with c-C_6H_{12} while its species **1d** is reactive ([*K*], 1.9). The same is true for $Fe^{II}(OPPh_3)_4^{2+}$ and $Cu^{I}(tpy)_2^{+}$.

In the presence of O_2 the species **1** that are formed by the complexes of Table 1 (ML_x/HOOH, equation 1) bind O_2 to give **1**(O_2) (species **5**), which react with hydrocarbon substrates to transform methylenic carbon centers (–CH_2–) to ketones.[5,6] The hydroxylation systems of Table 1 also produce significant amounts of ketone via the initial formation of **1d**. However, whereas species **1** are able to bind O_2 to give **5** as the reactive intermediate for ketonization (oxygenated Fenton chemistry), this is unreasonable for the various species **1d** (coordinately saturated as formulated in equation 9). This conclusion is supported by the much larger [*K*] values for the ketonization of c-C_6H_{12} by the hydroxylation systems (Table 1) relative to those for oxygenated Fenton chemistry (**5**) {e.g. with $Fe^{II}(PA)_2$, [*K*] = 7 (hydroxylation) vs 2.5 (O_2-Fenton)}. Although species **5** reacts with hydrocarbon substrates (RH) to give an alkyl peroxy intermediate (**6**),[6]

$$\underset{\mathbf{5}}{[(PA)_2Fe^{III}(OOH(BH^+))(OO\cdot)]} + \text{c-}C_6H_{12} \xrightarrow[B,\ H_2O]{} \underset{\mathbf{6}}{[(PA)_2Fe^{IV}(OH)(OOC_6H_{11})]} \quad (10)$$

species **1d** appears to form an analogous intermediate (**6d**)

$$\underset{\mathbf{1d}}{[(PA)_2Fe^{IV}(OOH)(DH)]} + \text{c-}C_6H_{12} + O_2 \xrightarrow[D,\ H_2O]{} \underset{\mathbf{6d}}{[(PA)_2Fe(OH)(OOC_6H_{11})]} . \quad (11)$$

Because species **6** reacts either with a second substrate molecule or the catalyst [$Fe^{II}(PA)_2$] to form ketone and either alcohols or oxidized catalyst,[6] species **6d** should have analogous reactivity. However, for the conditions of a hydroxylation system species **6d** will preferentially react with excess reductant (DH_2) to give the reaction stoichiometry of equation 8.

Scheme I outlines a set of proposed reaction paths that are in accord with these considerations, and the product profiles and reactivity parameters of Table 1.

For large DH_2:O_2 ratios the hydroxylation reaction (path **A**, Scheme I) is the dominant substrate transformation, but DH_2 is a competitive reactant (the reaction efficiency for c-C_6H_{12} with 50 mM PhNHNHPh is 30 %; Table 2). When the DH_2:O_2 ratio approaches unity the ketonization [or hydroperoxidation for olefins (c-C_6H_{10} → c-C_6H_9OOH)] reaction (path **B**, Scheme I) becomes competitive (or dominant) and the overall reaction efficiency approaches 100 % (Table 2).

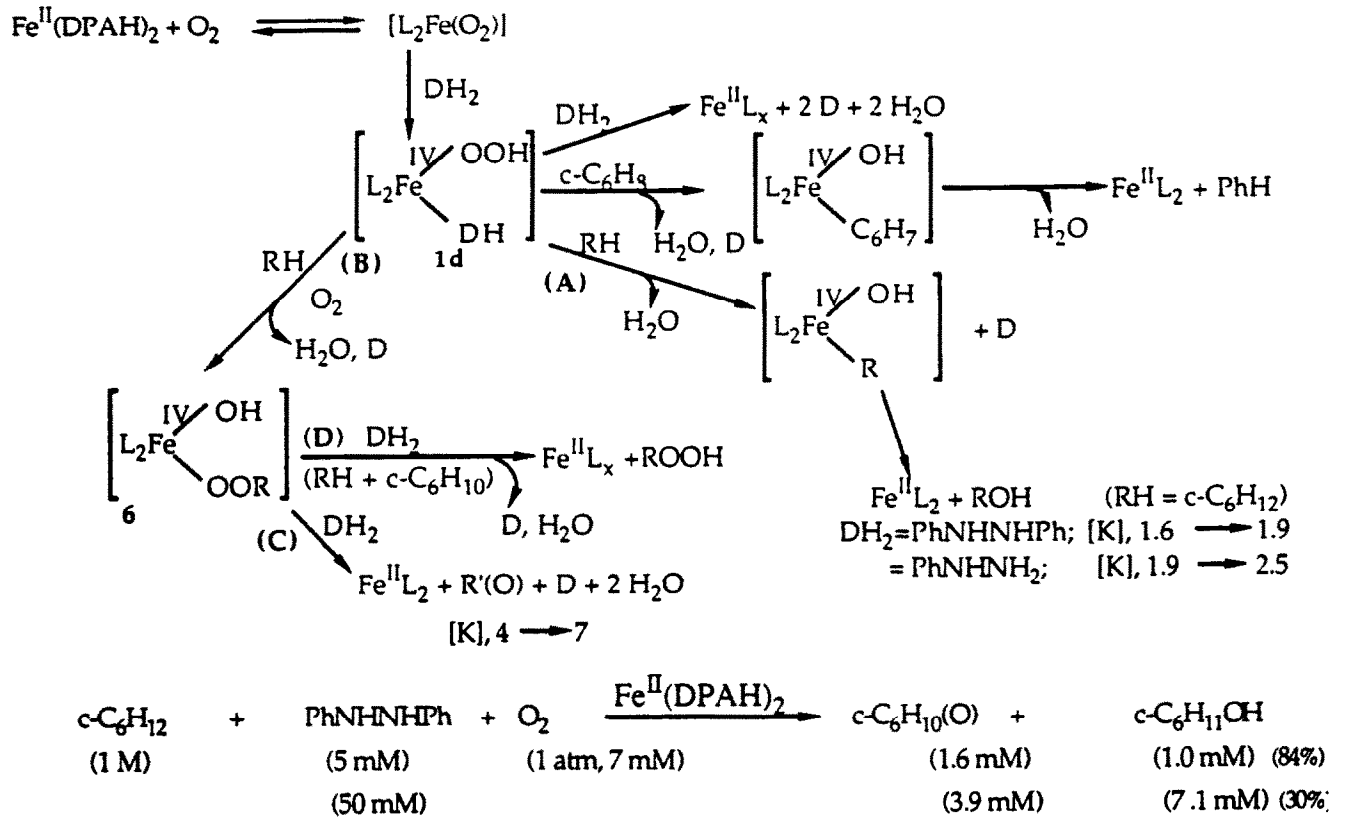
Iron-porphyrin catalysts

The activation of O_2 by the iron(III)-porphyrin [(por)$Fe^{III}X$]/ DH_2 combination (Table 1) appears to occur via the same pathways as the other iron and copper complexes (Scheme I). However, the initial step is preceded by reduction of the porphyrins

$$2\ (por)Fe^{III}X + DH_2 \rightarrow 2\ (por)Fe^{II} + D + 2\ HX. \quad (12)$$

The (Cl_8TPP)$Fe^{III}Cl$/O_2/PhNHNHPh/MeCN system is the most efficient and selective of the group under investigation for the hydroxylation of hydrocarbons. Only the iron-porphyrin catalysts, in their reaction with cyclohexene (c-C_6H_{10}), yield detectable amounts of alcohol (c-C_6H_9OH); the dominant product in all cases (Table 1).

With the model for the cytochrome P-450 hydroxylase proteins [(Cl_8TPP)$Fe^{III}SCH_2Ph$],[22] the results of Table 1 indicate that it is equivalent to the other porphyrins and, therefore, must be reduced via equation (12) prior to binding O_2 to form **1d** and react via paths **A** and **B** of Scheme I. When $PhCH_2SH$ is used as the reductase the (Cl_8TPP)$Fe^{III}SCH_2Ph$ complex is much more effective than the other complexes. This can be rationalized



Scheme I. $Fe^{II}(DPAH)_2$/reductant (DH_2)-induced activation of O_2 for the oxygenation of hydrocarbons.

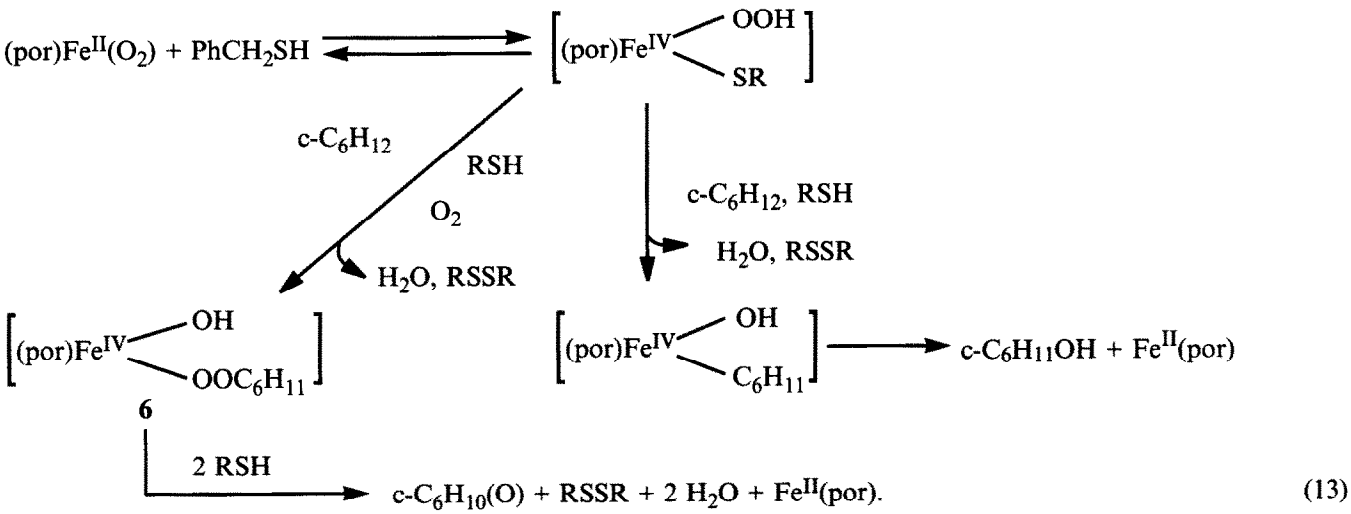
on the basis of a reasonable reaction sequence of the unique catalyst/reductant combination.

Extrapolation of the mechanistic proposals for the present model system in its aprotic matrix to cytochrome P-450 proteins in a biological matrix yields a self-consistent, reaction sequence (equation 14).

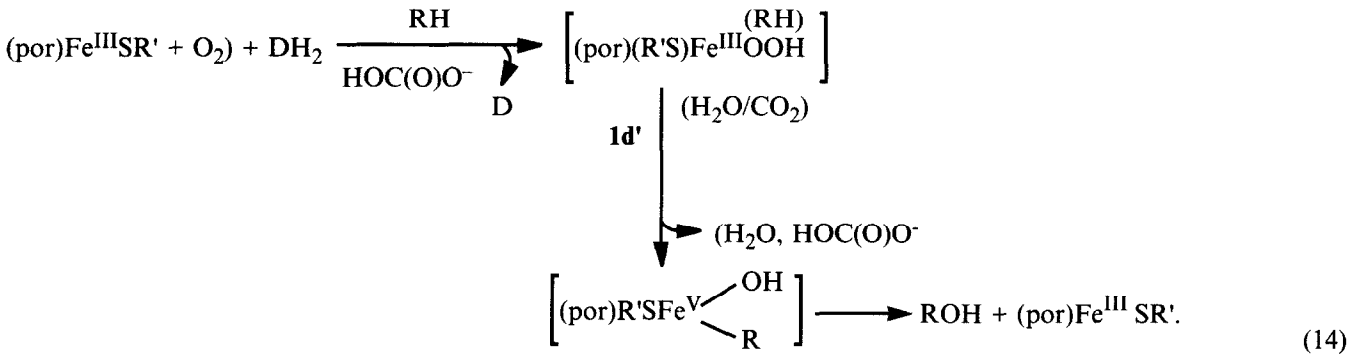
This suggested mechanistic path includes a reactive intermediate **1d'** that would favor hydroxylation of hydrocarbon substrates (including cyclohexene), which is in accord with the results for the cytochrome P-450/NADPH system under biological conditions.[23]

Cyclohexene ketonization and peroxidation

When cyclohexene ($c\text{-}C_6H_{10}$) is the substrate, the dominant product is the ketone (with no detectable alcohol) for all systems except (por)$Fe^{III}X$/PhNHNHPh, (por)$Fe^{III}SCH_2Ph$/$PhCH_2SH$, and $Fe^{II}(DPAH)_2$/$PhCH_2SH$ (Table 1). Thus, path **B** of Scheme I is favored for allylic carbon centers to form intermediate **6**, which can react with a second DH_2 via path **C** to form ketone or via path **D** to form the hydroperoxide ($c\text{-}C_6H_9OOH$). With these systems, the formation of hydroperoxides is limited to allylic carbon centers. If the reductant is $PhNHNH_2$, no hydroperoxide is produced from cyclo-



(13)



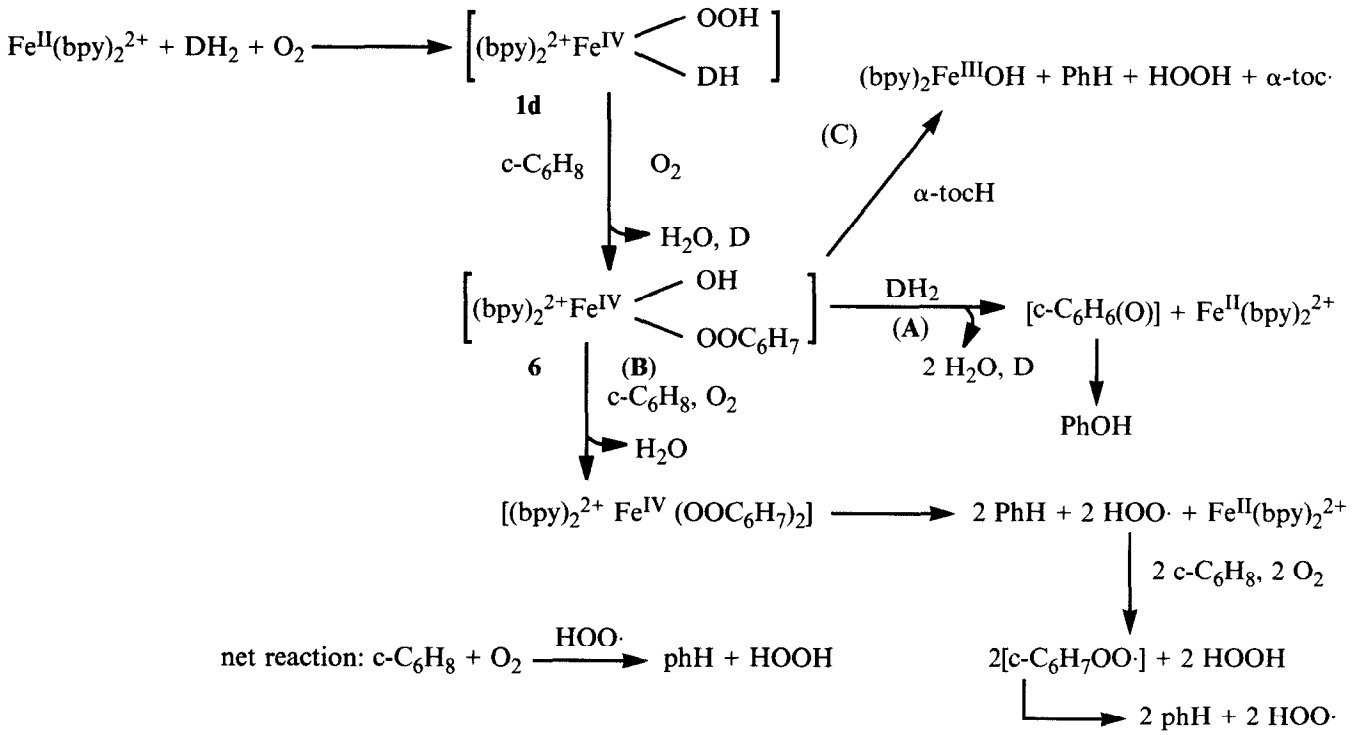
hexene (Table 1B). The inability to produce any epoxide from cyclohexene precludes reactive intermediates like compound I [(por$^{+\cdot}$)Fe^{IV}=O] of *horseradish peroxidase* and the ferryl group [L_xFe^{IV}=O, L_xFe^{V}=O]. Again, these systems react with olefins to yield product profiles that are similar to those for oxygenated Fenton chemistry,[5,6] but the latter systems only yield ketone.[24]

$Fe^{II}L_x/DH_2$-induced autoxidation of 1,4-cyclohexadiene (c-C_6H_8)

Although 1,4-cyclohexadiene (c-C_6H_8) (with two double allylic carbon centers) readily undergoes dehydrogenation to give benzene (PhH), substantial amounts of phenol (PhOH) are formed via the initial ketonization of an allylic carbon (Table 1).[19] When the reductant (DH_2)/catalyst (FeL_x) ratio approaches unity, several systems initiate the autoxidation of this substrate to form PhH (with O_2/DH_2 turnover numbers of 50–70, Table 3). Other catalyst/reductant combinations fail to exhibit any autoxidation. The addition of vitamin E (α-tocopherol) to the active autoxidation initiators reduces the turnover numbers by 25–80 %.

The initial step appears to be formation of **1d**, which reacts with c-C_6H_8 and O_2 to form intermediate **6** (Scheme II).[5,6]

The latter reacts with a second DH_2 via path **A** to give ketone, which enolizes to PhOH. With 1:1 $Fe^{II}(bpy)_2^{2+}$/$PhNHNH_2$ (or H_2asc) or $Fe^{II}(PA)_2/H_2$asc intermediate **6** reacts with a second c-C_6H_8 and O_2 to form [$(bpy)_2^{2+}Fe^{IV}(OOC_6H_7)_2$] via path **B**. The latter dissociates to give PhH and HOO·, which carries the autoxidation chain for up to 70 turnovers (Scheme II and Table 3). The presence of 10 mM α-tocopherol with the 5 mM $Fe^{II}(bpy)_2^{2+}$/5 mM $PhNHNH_2/O_2$/1 M c-C_6H_8 system reduces the yield of PhH from 355 mM (71 turnovers) to 67 mM (13 turnovers). This antioxidant may be a competitive substrate for species **6** (via path **C** of Scheme II) and thereby interfere with initiation of the autoxidation chain. Alternatively, α-tocopherol can react with HOO· (the carrier of autoxidation) to shorten the chains



Scheme II. $Fe^{II}(bpy)_2^{2+}$/reductant (DH_2; $PhNHNH_2$ or H_2asc))/O_2(1 atm)-initiated autoxidation of 1,4-cyclohexadiene (c-C_6H_8).

$$HOO\cdot + \alpha\text{-tocH} \longrightarrow HOOH + \alpha\text{-toc}\cdot \quad (15)$$

Iron-induced autoxidation of $PhCH_2SH$

Of the several catalysts studied, $Fe^{II}(OPPh_3)_4^{2+}$ is the most effective activator of O_2 for reaction with $PhCH_2SH$ (Table 5). Apparently this thiol reacts as a combined reductant and substrate. A reasonable set of reaction paths is outlined in Scheme IIIA. Although $PhCH_2SSCH_2Ph$ is the dominant product, with smaller $PhCH_2SH/O_2$ ratios formation of PhCH(O) becomes favored. When all of the $PhCH_2SH$ has been oxidized the PhCH(O) will undergo autoxidation to PhC(O)OH.[25]

In contrast, the $Fe^{II}(DPAH)_2$ complex is essentially inert towards the autoxidation of $PhCH_2SH$. However, in the presence of cyclohexene (c-C_6H_{10}, 1 M) a significant fraction of the $PhCH_2SH$ reductant is transformed to $PhCH_2SSCH_2Ph$, PhCH(O), and PhC(O)OH (Table 5). The extent of this substrate/reductant reaction is solvent dependent with 3:1 MeCN:pyridine the optimum (Table 4); about 60 % of the $PhCH_2SH$ is transformed. The production of acidic products [PhC(O)OH] probably inhibits O_2 binding by the catalyst and thereby terminates the reductant/substrate reaction. Scheme IIIB outlines a set of reaction paths for the $Fe^{II}(DPAH)_2/PhCH_2SH/O_2$/c-$C_6H_{10}$ system that are consistent with the product profiles of Tables 4 and 5.

Oxygen toxicity

The present iron/O_2/reductant systems appear to be closely similar to the presence of dysfunctional transition metals in combination with biological reductants in aerobic biology. The latter can reasonably be expected to activate O_2 for reaction with organic substrates (including saturated hydrocarbons) in an analogous manner. The level of activation should be similar to that for Fenton reagents,[5] oxygenated Fenton reagents,[6] and cytochrome P-450 hydroxylases. Although Fenton chemistry is relevant, there is no evidence from the product profiles or the reactivity parameters for free oxy-radicals (HO·, HOO·/$O_2^{-\cdot}$) or free carbon-radical intermediates. The efficient and selective reactivity of **1d** (Scheme I) (hydroxylation chemistry) and **6** (oxygenated hydroxylation chemistry) makes them more reasonable cytotoxic agents than free HO· and HOO·/$O_2^{-\cdot}$ within the oxy-radical theory of aging and heart disease.[12,13] Hence, dysfunctional transition metals, reductants, and O_2 are believed to be a hazardous combination in biology via the processes of Schemes I–III.

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 gas chromatography–mass spectrometry (Hewlett-Packard 5790A Series gas chromatograph with a mass-selective detector). A Vacuum Atmospheres inert-atmosphere glovebox was used for the storage, preparation, and addition of air-sensitive and water-sensitive reagents.

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. High-purity argon gas was used to deaerate the solutions. All compounds were dried *in vacuo* over $CaSO_4$ for 24 h prior to use. Ferric chloride (anhydrous, 98 %), picolinic acid (PAH, 99 %), 2,6-pyridinedicarboxylic acid ($DPAH_2$, 99 %), 2,2'-bipyridine (bpy, 99+ %), 2,2'-6,2"-terpyridine (tpy), and triphenylphosphine oxide ($OPPh_3$, 98 %) were obtained from Aldrich and hydrogen peroxide (50 % H_2O) was obtained from Fisher. Tetra(phenyl)porphinato-iron(III)-chloride [(TPP)Fe^{III}Cl] was obtained from Strem Chemical. The organic substrates included: cyclohexane (Aldrich, anhydrous, 99+ %), cyclohexane-d_{12} (Aldrich, 99.5 atom % D), cyclohexene (Fisher, 99 %), and 1,4-cyclohexadiene (Aldrich 96 %).

Syntheses of $(Me_4N)PA$ and $(Me_4N)DPAH$. Tetramethylammonium picolinate [(Me_4N)PA] and tetramethylammonium dipicolinate [(Me_4N)DPAH] were prepared by the neutralization of picolinic acid (PAH) and 2,6-pyridine-dicarboxylic acid ($DPAH_2$) with tetramethylammonium hydroxide pentahydrate in 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.

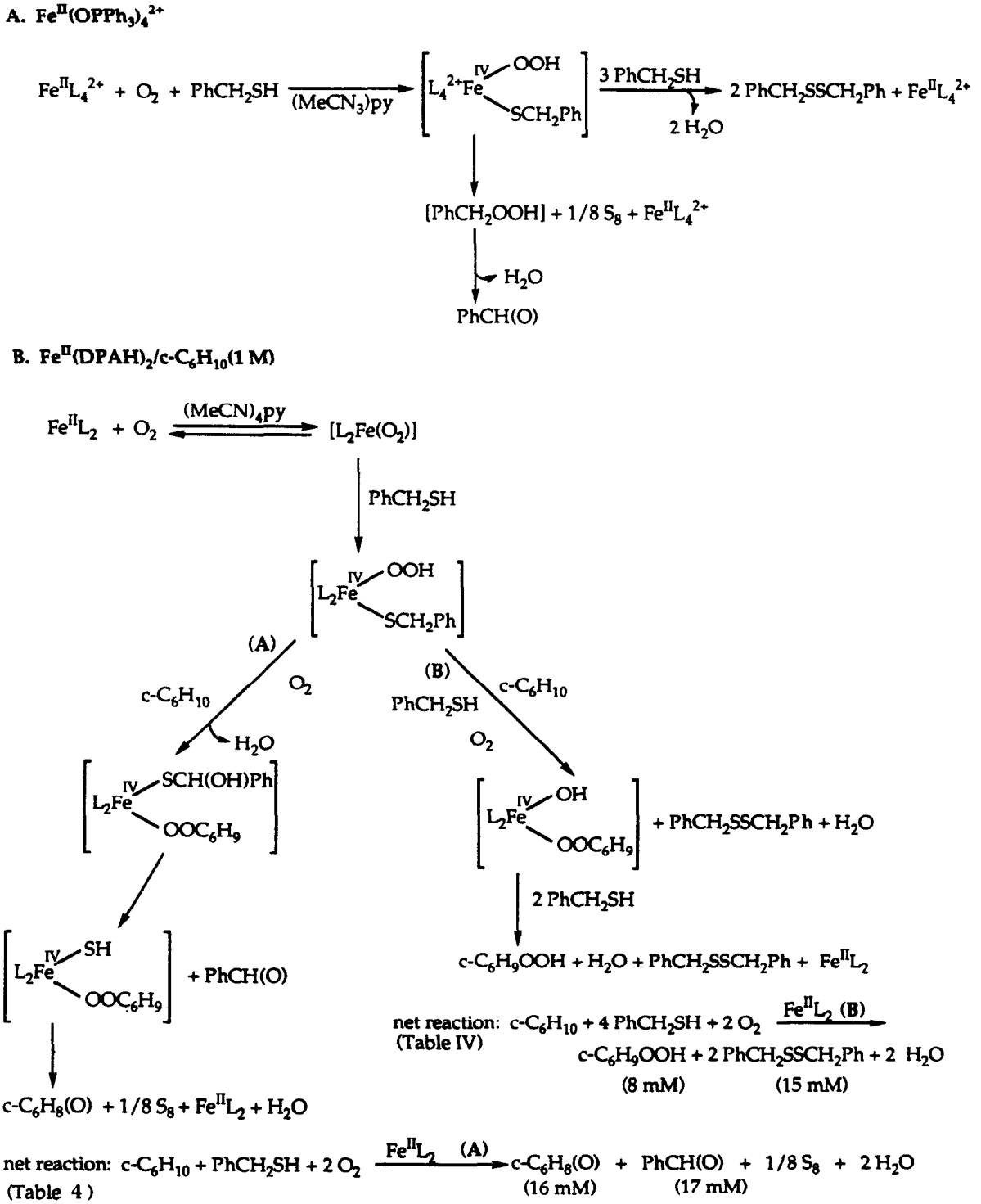
$[Fe^{II}(MeCN)_4](ClO_4)_2$. The $[Fe^{II}(MeCN)_4](ClO_4)_2$ complex was prepared by multiple recrystallizations of $[Fe^{II}(H_2O)_6](ClO_4)_2$ from MeCN.

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.[15]

Iron(II)bis(2,2'-bipyridine) and copper(I)bis(2,2'-6,2"-terpyrine) solutions. The $Fe^{II}(bpy)_2^{2+}$ and $Cu^{I}(tpy)^{+}$ complexes were prepared *in situ* by mixing $[Fe^{II}(MeCN)_4](ClO_4)_2$ and $[Cu^{I}(MeCN)_4](ClO_4)$ in MeCN with stoichiometric ratios of bpy and tpy, respectively.

Iron(II) tetrakis-(triphenylphosphine oxide) solutions. The $Fe^{II}(OPPh_3)_4^{2+}$ complex was prepared *in situ* by mixing $[Fe^{II}(MeCN)_4](ClO_4)_2$ in MeCN with a stoichiometric ratio of the $OPPh_3$ ligand.

Tetra(2,6-dichlorophenyl)porphyrinato–iron(III) complexes [$(Cl_8TPP)Fe^{III}X$; X=Cl, OH, SCH_2Ph]. The free por-



Scheme III. Iron(II)-induced autoxidation of thiols.

phyrin [Cl_8TPPH_2], which was prepared by a modified procedure,[16] was used to synthesize $(Cl_8TPP)Fe^{III}Cl$[17] and $(Cl_8TPP)Fe^{III}OH$.[18] In MeCN a 25-fold excess of $PhCH_2SH$ transforms 1 mM $(Cl_8TPP)Fe^{III}OH$ to 1 mM $(Cl_8TPP)Fe^{III}SCH_2Ph$ (98 % complete). This was the procedure used to prepare test solutions of this catalyst.

Methods

The investigations of O_2/reductant (DH_2) activation by the iron and copper complexes (ML_x) used solutions that contained 1.0 M substrate (RH), 3 mM ML_x, and 50 mM DH_2 in 3–4 mL of solvent [3:1 MeCN:pyridine $(MeCN)_3py$ or MeCN]. 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; 17 mL of headspace). After 4 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 charac-

terized by GC–MS. Reference samples were used to confirm product identifications and to produce standard curves for quantitative assays of the product species.

The kinetic isotope effect [$K = k_H/k_D$] was determined with a 1:1 cyclohexane/cyclohexane-d_{12} mixture (0.5 M/0.5 M) as the substrate; the k_H/k_D ratios were determined from the product ratios of c-$C_6H_{11}OH$/c-$C_6D_{11}OH$ and c-$C_6H_{10}(O)$/c-$C_6D_{10}(O)$.

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.

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