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Selective dehydrogenation (oxidation) of 3,4-dimethoxybenzyl alcohol by a non-heme iron lignin-peroxidase reaction mimic

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In pyridine, bis(2,2'-bipyridine)iron(II) ($Fe(bpy)_2^{2+}$) activates hydrogen peroxide for the efficient and selective catalytic dehydrogenation (oxidation) of veratryl alcohol (model-substrate monomer for lignin; 3,4-$(MeO)_2PhCH_2OH$). Several other complexes ($Fe^{II}(OPPh_3)_2^{2+}$, $Fe^{II}(O_2bpy)_2^{2+}$, $Fe^{II}(MeCN)_2^{2+}$, $Fe^{II}(PA)_2$, $Fe^{III}Cl_3$) are effective catalysts for the dehydrogenation of veratryl alcohol and benzyl alcohol, but their selectivity (relative reactivity with 3,4-$(MeO)_2PhCH_2OH$ vs. $PhCH_2OH$) is less than the 6.1 ratio that is observed for the optimized $Fe^{II}(bpy)_2^{2+}/H_2O_2$/pyridine (py) system. The reactivities have been determined for several other methoxybenzyl alcohols that are model substrates for lignin (e.g., 4-$MeOPhCH_2OH$ and $(MeO)_3PhCH_2OH$).

Bis(2,2'-bipyridine)iron(II); Lignin peroxidase; Veratryl alcohol; Reaction mimic; Dehydrogenation; Hydrogen peroxide

1. INTRODUCTION

Lignin, the Earth's second most abundant plant product (after cellulose), is composed primarily of phenylpropanoid monomeric units that are interconnected by a complex array of stable carbon–carbon and carbon–oxygen bonds [1,2]. Although it represents a vast renewable resource of organic carbon [3], relatively few microorganisms can degrade lignin and its complex methoxy-aromatic components: the most efficient are the filamentous wood-rotting fungi, particularly the white-rot fungi (e.g. *Phanerochaete chrysosporium*) [4–6]. The latter contains a heme protein (lignin peroxidase, LP) [7–10] that activates H_2O_2 for the degradation of lignin.

The heme of LP is in the high-spin Fe(III) state and the fifth ligand of the pentacoordinate iron center is a histidine residue [11–13]. During a catalytic cycle LP activates H_2O_2 in a manner that is similar to that for horseradish peroxidase (HRP) to give a compound I intermediate $[(Por^{+\cdot})Fe^{IV}=O]$. The degradation of lignin is believed to be initiated by LP compound I via the removal of an electron from one of the polymer's methoxylated aromatic rings to form a reactive cation radical center that undergoes spontaneous degradation reactions [14–17]. The latter include cleavage of the arylpropane side chains (C_α–C_β cleavage), ether-bond cleavage, aromatic-ring opening, hydroxylation, demethoxylation, oxidation of benzylic alcohols, formation of phenols and quinones, and carboxylic acid formation [9,14–19].

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Most LP model studies have used iron- and manganese–porphyrin complexes with alkyl hydroperoxide, sodium hypochlorite, potassium monopersulfate, or molecular oxygen and an electron source [19–26]. A thiol-mediated manganese peroxidase system oxidizes veratryl alcohol (3,4-$(MeO)_2PhCH_2OH$), anisyl alcohol (4-$MeOPhCH_2OH$), and benzyl alcohol ($PhCH_2OH$) (common model substrates for lignin degradation modeling to yield the corresponding aldehydes and coupled dimers) [24]. With LP, veratryl alcohol is readily dehydrogenated (oxidized) to its aldehyde, but benzyl alcohol is unreactive [9,18].

Here we report the development of an efficient and selective model system for LP. The combination of hydrogen peroxide (H_2O_2) with bis(2,2'-bipyridine)iron(II) (and related iron complexes) in a pyridine (py)-containing solvent produces a reactive intermediate that dehydrogenates 3,4-$(MeO)_2PhCH_2OH$ to give its aldehyde with 86% efficiency (with respect to H_2O_2), but is only 21% efficient in its reactivity with $PhCH_2OH$. This selectivity closely parallels that of LP with these substrates [9,18].

2. EXPERIMENTAL

2.1. *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).

2.2. *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

Table I

Relative reactivities for ML_x/100 mM H_2O_2/solvent systems for the dehydrogenation of 0.8 M 3,4-$(MeO)_2PhCH_2OH$ and 0.8 M $PhCH_2OH$[a]

Catalyst/solvent	Concentration (mM)	Yield of RCH(O) (mM, ±5 %)		
		3,4-$(MeO)_2$PhCH(O)	PhCH(O)	Ratio
$Fe^{II}(bpy)_2^{2+}$/py	1	67	39	1.7
/py	20	90[b]	20	4.5
/py	100	86	21	4.1
/py (200 mM H_2O_2)	100	134[c]	22	6.1
/MeCN	5	21	29	0.7
$Fe^{II}(OPPh_3)_4^{2+}$/py	1	70	34	2.1
/py	20	57	15	3.8
/MeCN	5	27	27	1.0
$Fe^{II}(MeCn)_4^{2+}$/py	5	58	22	2.6
/MeCN	5	16	31	0.5
$Fe^{II}(PA)_2$/$(py)_4$HOAc	5	63	46	1.4
/$(py)_2$HOAc	5	50	29	1.7
$Fe^{III}Cl_3$/py	1	63	27	2.3
/py	5	67	24	2.8
/py	20	55	16	3.4

bpy, pyridine; py, pyridine; MeCN, acetonitrile; HOAc, acetic acid

[a] Substrate and metal complex were combined in 7 ml of solvent, followed by the addition of 48 μl of 17.3 M H_2O_2 to give 100 mM H_2O_2. Results are the mean of duplicate or triplicate analyses.

[b] Plus 35 mM 3,4-$(MeO)_2PhC(O)OCH_2Ph(OMe)_2$-3,4; the presence of 100 mM $HClO_4$ results in 107 mM RCH(O) and 7 mM 3,4-$(MeO)_2PhC(O)OCH_2Ph(OMe)_2$-3,4.

[c] Plus 13 mM 3,4-$(MeO)_2PhC(O)OCH_2Ph(OMe)_2$-3,4.

(MeCN, 0.004% H_2O), and pyridine (py, 0.014% H_2O), were used as solvents. High-purity argon gas was used to de-aerate the solutions. 2,2′-Bipyridine (bpy, 99+%) was obtained from Aldrich; hydrogen peroxide (50% H_2O) from Fisher; *tert*-butyl hydroperoxide (5.5 M in 2,2,4-trimethylpentane) from Aldrich; and perchloric acid (A.C.S. reagent, 70%) from Mallinckrodt. The organic substrates included: 3,4-dimethoxybenzyl alcohol (veratryl alcohol, Aldrich, 96%), 4-methoxybenzyl alcohol (*p*-anisyl alcohol, Aldrich, 98%), benzyl alcohol (Aldrich, 99+%), 2-methoxybenzyl alcohol (*o*-anisyl alcohol, Aldrich, 99%), 3-methoxybenzyl alcohol (Aldrich, 98%), 2,3,4-trimethoxybenzyl alcohol (Aldrich, 90%), and 3,4,5-trimethoxybenzyl alcohol (Aldrich, 97%).

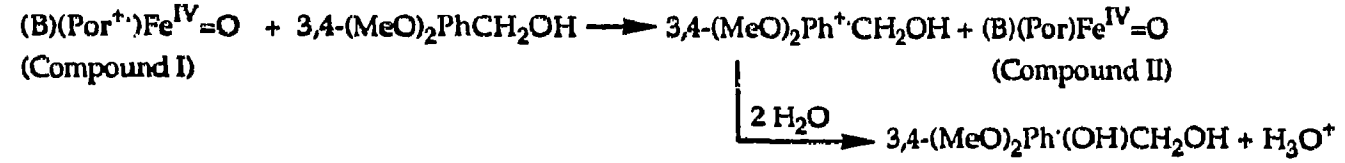
The $Fe^{II}(bpy)_2^{2+}$ complex was prepared in situ by mixing $[Fe^{II}(MeCN)_4](ClO_4)_2$ in MeCN with stoichiometric ratios of bpy.

2.3. *Methods*

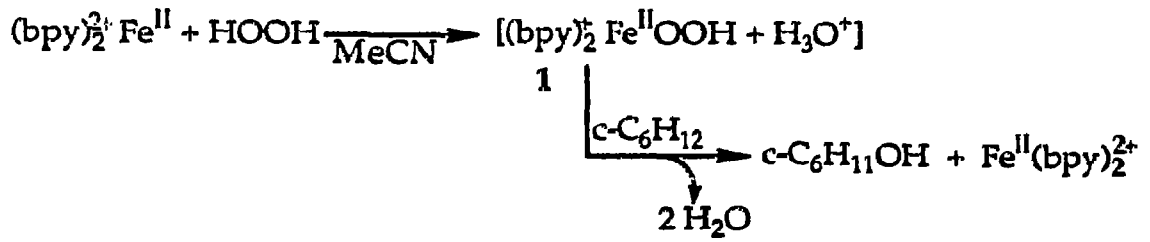
The investigations of H_2O_2 activation by $Fe^{II}(bpy)_2^{2+}$ used solutions that contained 0.8 M substrate and 20 mM metal complex in 7 ml of py. Hydrogen peroxide (50% wt/wt in H_2O) was injected to give 100 mM H_2O_2. After 4–6 h with constant stirring at room temperature (22 ± 2°C) under anaerobic conditions, samples of the reaction solutions were injected into a capillary column gas chromatograph for analysis. Product species were characterized by GC-MS.

3. RESULTS

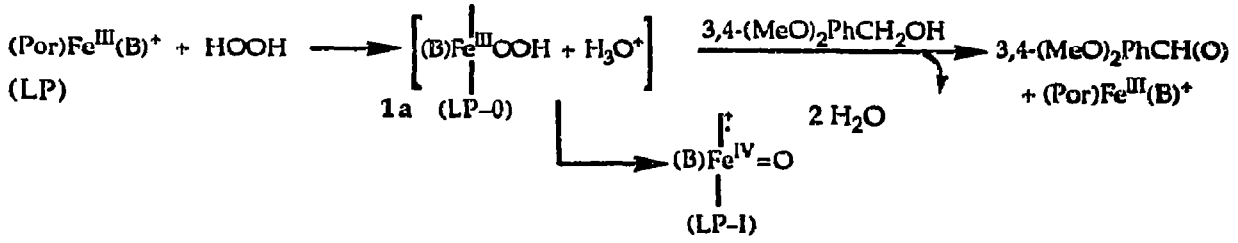
The product yields from the activation of hydrogen peroxide by iron complexes for reaction with 3,4-$(MeO)_2PhCH_2OH$ and $PhCH_2OH$ in three solvent matrices are summarized in Table I. The presence of py in the solvent increases the yield of 3,4-$(MeO)_2$PhCH(O) and diminishes the yield of PhCH(O), and thereby enhances the selectivity for reaction with 3,4-$(MeO)_2PhCH_2OH$. The $Fe^{III}Cl_3$ complex in py produces a high yield of 3,4-$(MeO)_2$PhCH(O) (67 mM) and moderate selectivity (ratio, 2.8). The $Fe^{II}(PA)_2$ and $Fe^{II}(DPA)_2^{2-}$ complexes in 4:1 py/HOAc are efficient cat-



Equation 1



Equation 2.



Equation 3.

alysts (yield of 3,4-$(MeO)_2PhCH(O)$; 63 mM), but have limited selectivity (ratio, 1.4). In MeCN the complexes produce reactive intermediates from H_2O_2 that exhibit a reversal of selectivity (i.e. they are more reactive with $PhCH_2OH$ than 3,4-$(MeO)_2PhCH_2OH$).

Variation of the catalyst/solvent systems indicates that 20 mM $Fe^{II}(bpy)_2^{2+}$ in pyridine is highly efficient, with a selectivity ratio of 4.5 (Table I). If the $Fe^{II}(bpy)_2^{2+}$ concentration is increased to 100 mM, the production of the secondary product:

3,4-$(MeO)_2PhCH(O)$+3,4-$(MeO)_2PhCH_2OH \xrightarrow{[O]}$ 3,4-$(MeO)_2PhC(O)OCH_2Ph(OMe)_2$-3,4+$H_2O$

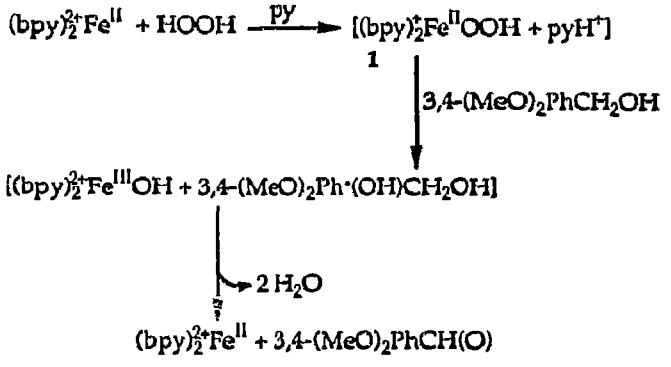
is suppressed and the selectivity ratio is 4.1. The 100 mM $Fe^{II}(bpy)_2^{2+}$/200 mM H_2O_2 system has the highest selectivity (ratio, 6.1), but 13 mM 3,4-$(MeO)_2$-$PhC(O)OCH_2Ph(OMe)_2$-3,4 is produced. The addition of protons to the pyridine solvent increases the reaction efficiency (107 mM 3,4-$(MeO)_2PhCH(O)$ produced) and suppresses ester formation.

4. DISCUSSION

Most investigators believe that Compound I [$(B)(Por^{+\bullet})Fe^{IV}=O$] of lignin peroxidase is the primary reactant with veratryl alcohol via electron transfer [14–17] (Eqn. 1). This mechanism is consistent with the relative ease of oxidation and reactivity of 3,4-$(MeO)_2PhCH_2OH$ ($E^{o'}_{ox}$,+1.65 V vs. NHE) and $PhCH_2OH$ ($E^{o'}_{ox}$,+2.24 V) [27]. However, the dominant product for the LP/veratryl alcohol system is 3,4-$(MeO)_2PhCH(O)$, which also is the case for the present ML_x/H_2O_2/py systems (Table I). Initial production of an aryl cation radical (Eqn. 1) should lead to a more diverse array of products, and the ML_x/H_2O_2 systems of Table 1 do not have the thermodynamic driving force (one-electron oxidation potential) to remove an electron from veratryl alcohol. Hence, the results of Table I must be due to another reaction path and reactive intermediate.

A recent study [28] of hydrocarbon hydroxylation by ML_x/H_2O_2 systems (analogous to those of Table I) provides compelling evidence that the initial activation step is a base-induced nucleophilic addition of H_2O_2 to the electrophilic metal center (Eqn. 2). Species **1** is able to abstract a hydrogen atom from c-C_6H_{12} (ΔH_{DBE}, 96 kcal · mol^{-1}), but not from ethane (ΔH_{DBE}, 100 kcal · mol^{-1}). When the substrate is $PhCH_3$, species **1** is 30-times more likely to add a hydroxyl group to the aromatic ring than to abstract a hydrogen atom from the methyl group. These considerations, and the product profiles and relative reactivities of the $Fe^{II}L_x/H_2O_2$ systems with 3,4-$(MeO)_2PhCH_2OH$ and $PhCH_2OH$ (Table I), prompt us to propose reaction pathways for the transformation of the substrates to their aldehydes (Scheme 1).

Scheme 1 is consistent with the greater reactivity of electron-rich 3,4-$(MeO)_2PhCH_2O$ relative to that of $PhCH_2OH$. Such enhanced electron density in the aromatic ring will favor electrophilic addition by a hydroxyl radical (HO•) from species 1. Furthermore,



Scheme 1.

(HO•) addition to the aromatic ring in preference to abstraction of a benzylic hydrogen atom is consistent with the observed reactivity of $PhCH_3$ with various species **1** [28]. All of which prompts us to suggest that LP activates H_2O_2 for the selective dehydrogenation of 3,4-$(MeOH)_2PhCH_2OH$ via a pathway that is analogous to that of Scheme 1 (Eqn. 3).

The combination of LP and H_2O_2 must initially form a precursor to its compound I (LP-I), which should have the form and reactivity of species **1** (Scheme 1). The formation of this precursor (**1a**, Eqn. 3) (sometimes referred to as compound 0 (LP-0)) for peroxidases has been discussed in terms of mechanism [29] and structure [30]. Species **1a**, which will be more reactive with electron-rich veratryl alcohol than with $PhCH_2OH$, reacts with the substrate via LP-0/dehydrogenation (Scheme 1 and Eqn. 3) and does not form compound I.

In summary, $Fe^{II}(bpy)_2^{2+}$ (and related iron(II) complexes) activates H_2O_2 in py-containing solutions to dehydrogenate veratryl alcohol with an efficiency (80–100%) and selectivity that closely parallels that of LP. The most reasonable pathway involves electrophilic addition of an (HO•) group from the reactive intermediate ($(bpy)_2^{\ddagger}Fe^{II}OOH+pyH^+$, species **1**) to the C-1 carbon of the aromatic ring (Scheme I). This is equivalent to the first two steps of the generally accepted electron-transfer mechanism for compound I of LP (Eqn. 1). The precursor to compound I (species **1a** (LP-0), Eqn. 3) should be as effective and as selective a reactant with veratryl alcohol as species **1** (Scheme 1), and appears to be a reasonable alternative as the primary reactant of LP.

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