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Modeling the Reactivity of α-Ketoglutarate-Dependent Non-Heme Iron(II)-Containing Enzymes

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α-Ketoglutarate-dependent Fe(II)-containing dioxygenase enzymes catalyze oxygenation of substrates by O_2 with concomitant oxygenation and decarboxylation of the cofactor to produce CO_2 and succinate.[1−4] Recently Chiou and Que have reported two studies in which Fe(II) complexes of benzoylformate (BF) were prepared and characterized as possible models for such enzymes.[5,6] Reaction of these complexes with O_2 resulted in conversion of the BF ligand to benzoate and CO_2, thus mimicking half of the typical dioxygenase reaction. However, simultaneous oxygenation (as opposed to oxidation) of substrates was not reported.[7] Before those reports appeared, we had obtained similar results using a different series of Fe(II) complexes of BF,[8] but in our case, the reaction with O_2 was much faster.[9] We attribute this difference in reactivity to the fact that the complexes of Chiou and Que contained tripodal tetradentate ligands and thus the metal centers in the bidentate BF complexes were six-coordinate, i.e. coordinatively saturated, whereas our complexes of facial tridentate ligands and BF were five-coordinate, leaving an open coordination position where O_2 might bind in a fashion similar to the reversible dioxygen binding complex reported for similar complexes by Kitajima et al.[10] We report here that an Fe(II) complex of BF and tris-(3,5-dimethyl-1-pyrazolyl)borohydride ($HB(3,5\text{-}Me_2pz)_3$) reacts with O_2 in the presence of olefinic substrates to give benzoate (from the BF ligand) and epoxides (from the olefinic substrates) in high yields. Thus this Fe(II) complex represents the first model of the α-ketoglutarate-dependent dioxygenases that displays simultaneous dioxygenation of an α-keto acid cofactor and an organic substrate by O_2.

$Fe^{II}(BF)(HB(3,5\text{-}Me_2pz)_3)(CH_3CN)$, **1**, was synthesized as follows. Anhydrous $FeCl_2$ was reacted with an equimolar amount of $HB(3,5\text{-}Me_2pz)_3$[11] in CH_2Cl_2, and the resulting complex was then reacted with sodium benzoylformate in acetonitrile to give **1** as a blue-purple, extremely air-sensitive solid.[12] The visible spectrum of **1** shows absorbances at 555 nm (sh) and 610 nm, giving it its characteristic color. The color and the visible spectrum of **1** are very similar to those previously reported for the bidentate BF complex of $Fe^{II}(TLA)$.[5,13] The ^1H NMR of **1** shows characteristic phenyl and boron hydride protons of the BF and $HB(3,5\text{-}Me_2pz)_3$ moieties.[14]

Table 1. Reaction of **1** and O_2 with Cyclohexene in the Absence and Presence of Sn Tributyl Hydride[a]

time (min)[b]	% yield[c] cyclohexene oxide	2-cyclohexene-1-one	2-cyclohexene-1-ol
	Without Sn Tributyl Hydride		
0	0	0	0
1.5	42	22	42
5	42	31	64
10	43	48	80
	With Sn Tributyl Hydride		
0	0	0	0
1.5	42	nd[d]	nd
5	42	nd	nd
10	43	nd	nd

[a] 20 mmol of cyclohexene and 0.11 mmol of Sn tributyl hydride (when indicated) were added to a solution of 20 mL of CH_3CN containing 0.1 mmol of **1** (prepared *in situ*) under inert atmosphere. The reaction was initiated by exposure to O_2 or air. All experiments were done at least in triplicate with a standard error of ±4%. [b] The reaction mixtures were analyzed for products by GC/MS at various times after O_2 or air exposure. [c] Yields are based on 0.1 mmol of **1**. Because **1** was prepared *in situ*, the actual yields may be higher. [d] Not detected.

Table 2. Reactions with Stilbenes[a]

substrate	% yield *cis*-stilbene oxide[f]	*trans*-stilbene oxide[f]	benzoic acid[g]
	Reactions with Compound **1**		
none	none	none	63
cis-stilbene[b]	65	trace	59
cis-stilbene[c]	50	trace	53
trans-stilbene[c]	trace	trace	65
cis-/trans-stilbene[d]	49	trace	55
	Reaction with MCPBA[e]		
cis-/trans-stilbene	80	20	

[a] All experiments were done at least in triplicate with a standard error of ±4%. [b] Reactions conditions: 10 mmol of *cis*-stilbene was added to a solution of 20 mL of CH_3CN containing 0.1 mmol of **1** (prepared *in situ*) under an inert atmosphere. The reaction was initiated by exposure to O_2 or air. [c] Same conditions except that 1 mmol of *cis*- or *trans*-stilbene was added. [d] Same conditions except that a mixture of 1 mmol of *cis*-stilbene and 1 mmol of *trans*-stilbene was added. [e] 0.1 mmol of MCPBA (*meta*-chloroperoxybenzoic acid) was added to a 20 mL solution of CH_3CN containing a mixture of 1 mmol of *cis*-stilbene and 1 mmol of *trans*-stilbene. Products were analyzed after 1 h. [f] Products were analyzed by HPLC. Yields are based on 0.1 mmol of **1** or 0.1 mmol of MCPBA. Because **1** was prepared *in situ*, the actual yields may be higher. [g] Products were analyzed by HPLC after addition of 0.5 mL of concentrated HCl. Yields are based on 0.1 mmol of **1**. Because **1** was prepared *in situ*, the actual yields may be higher.

Complex **1** was found to be highly reactive with either air or O_2 in acetonitrile and to give the same products in either case. Results of representative reactions are as follows. A solution of 0.1 mmol of **1** in 20 mL of acetonitrile[15] in a Schlenk tube

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(1) Hanauske-Abel, H. M.; Günzler, V. J. *Theor. Biol.* **1982**, *94*, 421−455.

(2) Thornburg, L. D.; Lai, M.-T.; Wishnok, J. S.; Stubbe, J. *Biochemistry* **1993**, *32*, 14023−14033.

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(4) Feig, A. L.; Lippard, S. J. *Chem. Rev.* **1994**, *94*, 759−805.

(5) Chiou, Y.; Que, L. *J. Am. Chem. Soc.* **1992**, *114*, 7567−7568.

(6) Chiou, Y.; Que, L. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1886−1888.

(7) Oxidation of 2,4-di-*tert*-butylphenol to give the corresponding biphenol was reported by Chiou and Que in ref 5.

(8) Kisiel, J. F. Master of Science Thesis, University of California, Los Angeles, 1990.

(9) Chiou and Que[5,6] reported reaction times of 2 days to 1 week, while our reactions were completed in 10−15 min.

(10) Kitajima, N.; Fukui, H.; Morooka, Y.; Mizutani, Y.; Kitagawa, T. *J. Am. Chem. Soc.* **1990**, *112*, 6402−6403.

(11) Niedenzu, K.; Trofimenko, S. *Top. Curr. Chem.* **1986**, *131*, 1−37.

(12) The solid was obtained in 35% yield and elemental analysis (Galbraith Laboratories) is consistent with **1**. Anal. Calcd: C, 55.28; H, 5.57; N, 18.05; B, 1.99; Fe, 10.28. Found: C, 55.60; H, 5.55; N, 17.99; B, 2.01; Fe, 10.02.

(13) The complex $Fe^{II}(BF)(TLA)$ displayed absorbances at 544 and 590 nm (sh) (see ref 5). Abbeviations: TLA, tris[(6-methyl-2-pyridyl)-methyl]amine.

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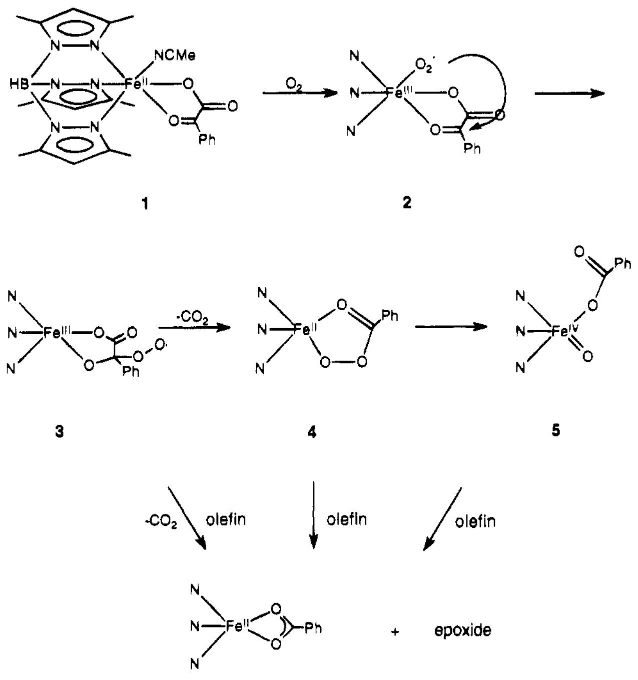
was exposed to air and stirred vigorously. Within 2 min, the initial blue-purple reaction mixture underwent 3 distinct color changes.[16] After 15 min, a small amount of aqueous HCl was added. The resulting solution was analyzed by HPLC and found to contain 0.07 mmol of benzoic acid. A similar reaction was carried out with excess cyclohexene (Table 1) or *cis*- or *trans*-stilbene (Table 2) present, and the products of the resulting solution were analyzed.

We chose initially to study olefin epoxidation rather than alkane hydroxylation because metal-mediated oxygen atom transfer to substrates can be more easily distinguished from free-radical autoxidation in the former case. Cyclohexene and *cis*-stilbene are particularly attractive substrates in this respect because free radical oxidation reactions typically lead to allylic oxidation of the former and *cis*−*trans* isomerization of the latter.

In the case of cyclohexene, three products were detected by GC−MS, cyclohexene oxide in 35−45% yield, based on the initial amount of **1**, and variable amounts of the allylic oxidation products, 2-cyclohexen-1-ol and 2-cyclohexen-1-one (Table 1). The amounts of the allylic products were found to increase with the time of reaction. We also carried out this reaction in the presence of tributyltin hydride[17,18] in order to determine if free radical autoxidation was taking place. We found that that the reaction pathway leading to allylic oxidation of cyclohexene was blocked in the presence of this free radical scavenger but that the pathway leading to epoxidation was unaffected (Table 1). In the case of *cis*-stilbene, *cis*-stilbene oxide in 50−65% yield and trace amounts of *trans*-stilbene oxide and benzaldehyde were detected (Table 2). The observation of cyclohexene epoxidation with no allylic oxidation and of *cis*-stilbene epoxidation with no *cis*−*trans* isomerization indicate that the predominate pathway for oxygenation is not free radical autoxidation.

Reaction of *trans*-stilbene gave only trace amounts of *trans*-stilbene oxide (Table 2). A mixture of *cis*- and *trans*-stilbenes was also reacted with **1** and with *m*-chloroperbenzoic acid as a control. Only the *cis*-oxide was observed in the former case, while both *cis*- and *trans*-oxides were observed in the latter (Table 2). The observation that *trans*-stilbene is unreactive under conditions where *cis*-stilbene is highly reactive suggests that the oxygen transfer to olefin takes place via a sterically congested transition state that will not permit *trans*-stilbene to approach closely enough for reaction to take place (see below).

Scheme 1



Scheme 1 describes possible reaction mechanisms which would be consistent with our results. The initial BF complex, **1**, is proposed to react with dioxygen, displacing the coordinated acetonitrile and forming a ferric superoxide complex, **2**. The superoxide ligand is proposed to react then with the coordinated BF ligand to form a peroxy adduct such as **3**. Complex **3** might then react directly with the olefin to give the observed epoxide product or decarboxylate to give the perbenzoate complex **4**. Alternative mechanistic possibilities would be the reaction of complex **4** with the olefin or reaction of a high-valent iron−oxo complex **5**, formed from the O−O bond cleavage in the coordinated perbenzoate complex **4**, with the olefin. Molecular modeling of complexes **3**−**5** indicates that *cis*-stilbene would be able to approach the iron center, with minimal steric hindrance, but that *trans*-stilbene would not. Presumably, the high degree of selectivity for *cis*- over *trans*-stilbene is due to the fact that the reactive intermediate generated in this stoichiometric reaction decomposes rapidly, in competition with the bimolecular reaction with the olefin, which, in the case of *trans*-stilbene, is slower.

In summary, complex **1** is the first model complex of α-ketoglutarate-dependent Fe(II)-containing dioxygenase enzymes that oxygenates both the α-keto cofactor and a substrate simultaneously. Future work will concentrate on elucidating the mechanism of this system, which may provide some insight into the mechanism of these dioxygenase enzymes.

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(14) A ^{1}H NMR spectrum of **1** in CD$_3$CN was obtained, and the peak assignments and integration are consistent with **1**. Data for the BF moiety: *o*-H at 22 ppm, *m*-H at 12 ppm, and *p*-H at 17 ppm. Data for the HB(3,5-Me$_2$pz)$_3$ moiety: methyl H at 66 ppm and boron H at 43 ppm.

(15) Because of the instability of **1**, it was prepared *in situ* for each reaction, by addition of equimolar amounts (0.1 mmol) of benzoylfomic acid, FeCl$_2$, and HB(3,5-Me$_2$pz)$_3$ in 20 mL of acetonitrile under He and Ar atmosphere. These solutions were then filtered and used in the subsequent reactions.

(16) Color changes as follows: Blue-purple to green to yellow to orange.

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