by data for the reduction of [C$_5$H$_5$N(C$_2$H$_5$)]Br in pyridine containing 0.1 M (TBA)PF$_6$ (Figure 6b). This compound has a single irreversible reduction process at E_p = -1.20 V and may be compared to a similar irreversible peak which is located at E_p = -1.21 V after the overall two-electron oxidation of (TPP)Co(C$_2$H$_5$)(py).

The formation of [(TPP)Co(py)$_2$]$^+$ as a porphyrin product after oxidation of (TPP)Co(C$_2$H$_5$)(py) in pyridine follows from analysis of current–voltage curves for the reduction and oxidation of [(TPP)Co(py)$_2$]$^+$ under similar experimental conditions. A cyclic voltammogram of (TPP)Co in pyridine is shown in Figure 8 and has reversible peaks located at $E_{1/2}$ = -0.25 and -1.01 V. These potentials are similar to the $E_{1/2}$ values of peaks IV and V in Figure 6 and are thus also consistent with the proposed oxidation/reduction mechanism shown in Scheme II.

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Oxidation of Alkanes by Cobalt(II) Salts of Weakly Coordinating Anions

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Catalysts which effect the selective oxidation of alkanes under mild reaction conditions are highly desired. Commercial processes exist which involve the oxidation of alkanes by O$_2$ with cobalt carboxylate catalysts. Elevated temperatures and pressures are required, and the metal ion function is to decompose hydroperoxides formed in a radical-chain process. We have demonstrated that a weakly solvated cobalt–acetonitrile complex, [Co(NCCH$_3$)$_4$](PF$_6$)$_2$, with a weakly coordinating anion catalyzes the air oxidation of alkanes under mild conditions (75 °C and 3 atm). Cyclohexane and adamantane are converted to the corresponding alcohol and ketone products. The commercial catalyst for cyclohexane oxidation does not function under these milder conditions. Experiments indicate a mechanism in which the metal ion functions both as an initiator and as a hydroperoxide decomposition catalyst.

Introduction

Autoxidation reactions tend to oxidize organic substrates via radical-chain processes leading to a nonselective product distribution.[1] However, selective, economically feasible autoxidation processes can be carried out with metal ion catalysts. Currently, cyclohexane is oxidized industrially using air under conditions of 165 °C and 10 atm.[2,3] The major products of this oxidation, cyclohexanol and cyclohexanone, referred to as KA oil, are precursors for the production of adipic acid (a precursor for Nylon 6,6). In view of the current interest in the activation of alkanes, the selective catalytic oxidation of cyclohexane under mild conditions is of fundamental and commercial significance.[4]

In this article we report that the cobalt–acetonitrile complex [Co(NCCH$_3$)$_4$](PF$_6$)$_2$ (in acetonitrile) is capable of catalyzing the oxidation of alkanes under mild conditions using O$_2$ as the primary oxidant. Under our reaction conditions (75 °C, 3 atm of air) cyclohexane is oxidized to a ~3:2 ratio of cyclohexanol and cyclohexanone. The catalyst used in the commercial process[5] for the oxidation of cyclohexane, Co(oct)$_2$ (oct = 2-ethylhexyl octanoate), is unreactive under our reaction conditions. We report experiments to determine if this novel reactivity involves a catalyst that is more effective at peroxide decomposition than the com-

mercial catalyst (initiating the chain reaction at lower hydroperoxide thresholds) or if a high oxidation state form of the metal complex reacts with the substrate to initiate the reaction.

Experimental Section

Materials. Adamantane (Aldrich), silver hexafluorophosphate (Aldrich), anhydrous cobalt(II) chloride (Alfa), cyclopentanone (Aldrich), benzene (Fisher), and ceric ammonium nitrate (Fisher) were used as received. Acetonitrile was stored over 4-Å molecular sieves prior to use. Cyclohexane was stored over P$_2$O$_5$ prior to use.

Cobalt–Acetonitrile Complex, [Co(NCCH$_3$)$_4$](PF$_6$)$_2$. The catalyst used for the oxidations described herein was prepared "in situ" in acetonitrile. Stoichiometric amounts of anhydrous CoCl$_2$ and AgPF$_6$ were combined in acetonitrile. The precipitated AgCl was filtered off. The resulting solution was tested for Ag$^+$ and Cl$^-$, since trace amounts of these ions decrease the activity of the catalyst. Blanks were performed using both AgCl and AgPF$_6$ in the absence of any cobalt. These resulted in no oxidation.

Physical Measurements. The NMR spectra were recorded on a multinuclear Varian VXR 300MHz spectrometer. The UV/vis spectra were obtained using a Perkin-Elmer Lambda 6 spectrometer. The products of the oxidation reactions were analyzed and quantified using a Varian 3300 gas chromatograph equipped with a FID detector and a chromosorb 15% DEGS column (6 ft). Further analysis of the reaction products were performed by GCMS using a Varian 3400 gas chromatograph interfaced with a Finnigan MAT ITDS 700 mass spectrometer. The column employed for this technique was a 15-m dbl column. Electrochemical studies were performed in acetonitrile with a PAR Model 175 universal programmer attached to a Model 173 potentiostat/galvanostat. Solutions of 0.05 M Co^{2+} and 0.1 M in tetrabutyl-ammonium hexafluorophosphate were employed.

Oxidations. Oxidations were carried out in a batch reactor using a 250-mL Parr pressure apparatus as previously described.[6] A standard reaction consisted of the following: catalyst (10^{-5} mol), acetonitrile (10

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mL), cyclopentanone (6 mL), cyclohexane (30 mL), and benzene (1 mL, GC standard). The pressure apparatus was placed into a constant-temperature oil bath and pressurized with air. The reaction solution was continuously stirred with a Teflon-coated magnetic stir bar during the reaction. Reactions were run for 12 h with samples taken periodically for analysis. For the adamantane oxidations, 10^{-2} mol of substrate was dissolved in acetonitrile. In these reactions, adamantane has only a limited solubility in acetonitrile.

Caution! Extreme caution should be taken when pressurizing cyclohexane with air at 75 °C. Appropriate shields must be used and fire extinquishers should be nearby. The reaction solution must be cooled before venting at the end of the reaction. The vapors and solution are *extremely flammable.*

Results and Discussion

Catalyst Characterization. The $[Co(NCCH_3)_4](PF_6)_2$ complex used in these studies was prepared "in situ" in acetonitrile. Due to the weakly coordinating nature of PF_6^-, this counteranion is easily displaced by acetonitrile, forming a solvated Co(II) ion-paired species. A series of ^{19}F NMR experiments indicate that PF_6^- is not in the inner coordination sphere of the metal center. Starting with 0.5 M solution of $[Co(NCCH_3)_4](PF_6)_2$ in acetonitrile, several ^{19}F NMR spectra were obtained at various concentrations. The spectra at 0.5 M showed a doublet due to PF_6^- (−69.8, −72.3 ppm). This doublet corresponds to that observed for $Na^+PF_6^-$ in acetonitrile. Successive dilutions to 0.1 and 0.001 M led to no observable shift in the PF_6^- resonance. Furthermore, the ^{19}F NMR spectra of the catalyst solution containing a standard reaction concentration of cyclohexane and cyclopentanone also show no observable shift in the PF_6^- resonance. Since coordination of PF_6^- to a paramagnetic cobalt(II) would lead to a large isotropic shift, these experiments strongly suggest that the catalyst in solution is a solvated cobalt(II) with the general formula $[Co(NCCH_3)_x]^{2+}$ and dissociated PF_6^- counterion.

UV/vis spectra of this complex were obtained at various concentrations to determine the geometry of the complex. A 0.01 M solution of the complex has a molar absorptivity of 495, characteristic of a tetrahedral geometry. Upon further dilution of the complex with acetonitrile to 10^{-3} and 10^{-4} M, the molar absorptivity decreased to 470 and 452, respectively. On the basis of the NMR results and these molar absorptivities, the predominant cobalt species in solution is the four-coordinate $[Co(NCCH_3)_4](PF_6)_2$ complex.

Catalyst Optimization. Although acetonitrile and cyclohexane are not miscible at 75 °C, a stirred solution of 1×10^{-5} mol of $[Co(NCCH_3)_4](PF_6)_2$ in acetonitrile at 3 atm of air leads to an active catalyst for the oxidation of cyclohexane. Under these conditions 1 turnover/h results. The biphasic nature of the solution suggests that the oxidation of cyclohexane occurs at the interface of the droplets that are formed as the solution is stirred. To help increase the solubility of the catalyst solution in cyclohexane, cyclopentanone is added to the reaction mixture. The addition of this ketone to the reaction mixture simulates commercial conditions. The commercial reaction is run by continuously withdrawing products and adding reactants. Accordingly, a steady-state concentration of KA oil (a mixture of cyclohexanol and cyclohexanone) is present in the commercial reactor for oxidation of cyclohexane.[3] Cyclopentanone was selected for our study, since it would not interfere with the GC analysis for cyclohexanone. This solution was found to be 25 times (25 turnovers/h) more active than the biphasic system and visually appears to be homogeneous. The increased activity may also result from radicals produced by the oxidation of cyclopentanone during the induction period. When $Co(oct)_2$ is used as the catalyst in this mixed-solvent system, no oxidation was observed in 12 h. When the reaction is carried out in the absence of the catalyst, no oxidation occurred in 18 h. The total reaction time employed for our metal-catalyzed oxidations was 12 h unless otherwise stated. As seen with the above blanks, this ensured the oxidation reactions were initiated by the metal complex. Experiments were also performed using this reaction mixture in the absence of any acetonitrile. No oxidation occurred in this sytem. The remaining experiments discussed here were carried out with acetonitrile and cyclopentanone.
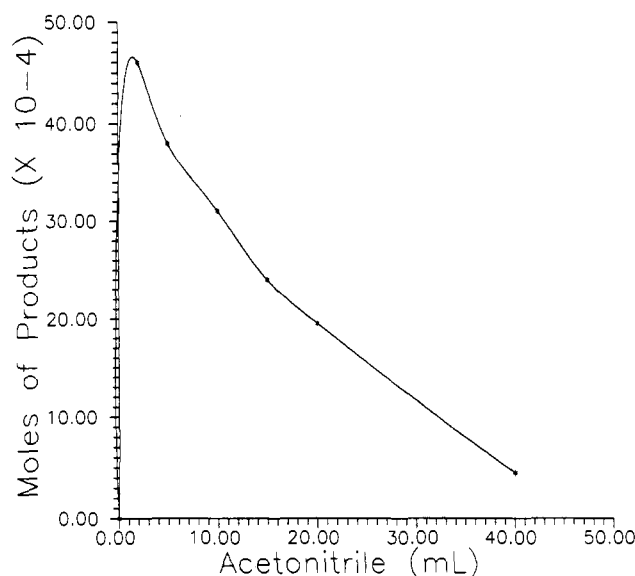

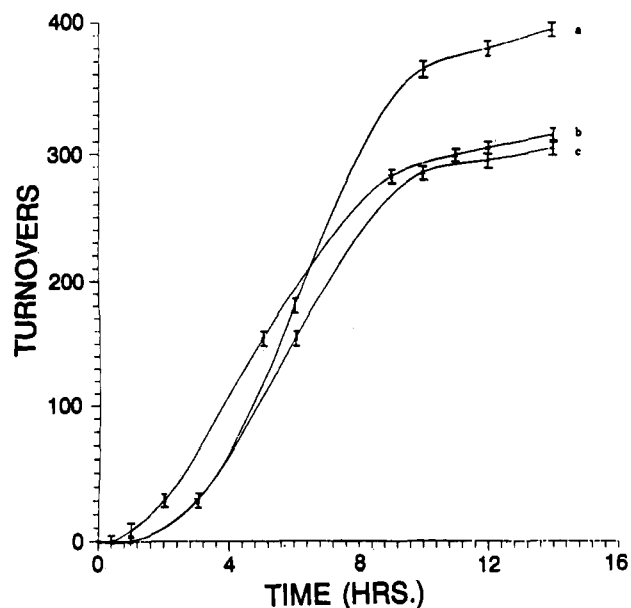**Figure 1.** Variation of acetonitrile in the oxidation of cyclohexane.

Figure 2. (a) Reaction of $[Co(CH_3CN)_4](PF_6)_2$ with cyclohexane with sieves. (b) Reaction $[Co(CH_3CN)_4](PF_6)_2$ prereacted with O_2 for 2 h prior to addition of cyclohexane. (c) Reaction of $[Co(CH_3CN)_4](PF_6)_2$ with cyclohexane.

To determine the effect the solvent has on the reaction, the volume of acetonitrile used in the reaction was varied. These experiments are illustrated in Figure 1. The results show that as the concentration of acetonitrile is decreased, while the amount of cobalt is held constant and a total volume of 50 mL is maintained, there is an increase in the moles of products formed after the standard 12 h of reaction time. When the volume of acetonitrile is decreased below 2 mL, a loss in activity is observed. This decrease is due to the insolubility of the acetonitrile catalyst solution in the cyclohexane/cyclopentanone solution. The results of these experiments indicate that this system is not a truly homogeneous solution. In a homogeneous system the volume of acetonitrile should have a minor dielectric effect on the overall activity, since the cobalt concentration in the total solution was constant in all cases. In a suspension, increasing the acetonitrile concentration would decrease the cobalt concentration on the surface of the droplet formed. We attribute the observed decrease in activity in our system to this phenomenon.

The use of solvents other than acetonitrile was also investigated. No activity was observed in propionitrile, THF, or propylene
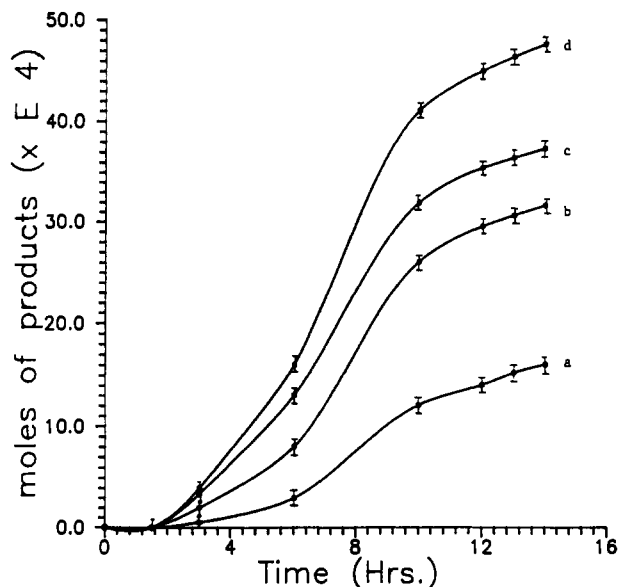
Figure 3. Variation of catalyst concentration for the oxidation of cyclohexane: (a) 1×10^{-6} mol; (b) 1×10^{-5} mol; (c) 2×10^{-5} mol; (d) 4×10^{-5} mol.

carbonate. Acetone, results in the same product distribution, although there is a 10-fold decrease in the moles of products formed in 12 h as compared to acetonitrile.

As seen in Figure 2, the formation of products ceases after 12 h of reaction time. After approximately 10 h, the initial blue catalyst solution turns pink, characteristic of an octahedral cobalt(II) complex. To determine if water, formed during the oxidation, is coordinating to cobalt(II) and inhibiting this reaction, the following experiments were carried out. After 6 h of reaction time, a small aliquot of water was added to the reaction mixture. The initially blue catalyst solution turned pink at once, and no further formation of oxidation products is observed. When 4-Å activated molecular sieves were added to the reaction mixture, prior to reaction, an increase in the moles of products was observed (Figure 2). Although the activated sieves can absorb 60% of their weight in water, the reaction stopped when only 25% of their weight in water was formed. The amount of water distributed between the solvent and the sieve is governed by a distribution coefficient. Hydrogen bonding of the water to acetonitrile results in an increased water concentration in the acetonitrile to the point where its concentration is large enough to hydrate cobalt(II) when only approximately 25% of the sieves total capacity for water is attained. The inhibition of the reaction by the water from the peroxide decomposition seen in our catalyst system is also reported with other cobalt complexes.[7,8] These experimental results indicate the formation of water, produced from the decomposition of the peroxides formed during the reaction, does inhibit the reaction. The use of acetonitrile dried over sieves, as well as the use of dried cyclohexane, also leads to an increase in the amount of products formed.

To further optimize this system, the catalyst concentration was varied (Figure 3). Although doubling the catalyst concentration to 2×10^{-5} mol resulted in an increase in products formed, a doubling in moles of products was not observed. Using this concentration of catalyst, 3.6×10^{-3} mol of alcohol and ketone was obtained. Increasing the catalyst concentration to 4×10^{-5} mol resulted in 4.7×10^{-3} mol of products being formed. When the catalyst concentration is decreased to 5×10^{-6} mol, the products formed decrease by 80% from that of 1×10^{-5} mol of catalyst, yielding 6.3×10^{-4} mol of product. From these ex-

Table I. Selectivity Study

catalyst, mol	reaction time, h	% selectivity	% conv
10^{-5}	12	50	5
10^{-5}	8	75	4
10^{-6}	12	91	1

periments, 1×10^{-5} mol of catalyst seems to be the optimum catalyst concentration for these reaction conditions.

Our next concern is in evaluating the selectivity of our catalyst (see Table I). The total oxygenated products formed were determined by capillary GC using standard silation techniques.[9] Using 10^{-5} mol of our catalyst results in a 50% selectivity toward cyclohexanol and cyclohexanone and proceeds with a 5% conversion of the cyclohexane to total products. In the industrial process,[3] a 3–5% conversion with 70% selectivity is obtained. The conversion of this reaction is kept low to prevent the further oxidation of the alcohol and ketone to undesirable ring-open products. A large range of other products is commonly observed in the oxidation of cyclohexane.[4] These products range from dicarboxylic acids to lactones. By a decrease of the reaction time from 12 to 8 h, an increase in selectivity to 75% is obtained, with a decrease to 4% conversion. This increase in selectivity results from less of the alcohol and ketone being further oxidized. By a decrease of the catalyst concentration to 10^{-6} mol, 91% selectivity to the alcohol and ketone is observed, but a large decrease to 1% conversion was observed.

Oxidation with $[Co(NCCH_3)_4](PF_6)_2$ was next carried out under the more stringent conditions used industrially (165 °C and 10 atm) to determine the effect on the conversion and selectivity. Running the reaction using 10^{-6} mol of catalyst resulted in a substantial decrease in selectivity from 91% to 34%, although an increase in the total conversion to 5% was observed. Thus, under these more stringent conditions, 10^{-6} mol of our catalyst gives conversions comparable to 10^{-5} mol of catalyst at 75 °C. This increased activity of our cobalt catalyst suggests that even lower concentrations of catalyst would be optimal at high temperatures and pressures.

Mechanistic Studies. Various experiments were carried out on the catalyst system to investigate the mechanism of the reaction using $[Co(NCCH_3)_4](PF_6)_2$. The addition of a free-radical inhibitor, benzoquinone, to the reaction results in no products being observed. Adding AIBN (a free-radical initiator, azobis(isobutyronitrile) to the acetonitrile solution containing the cobalt complex had no effect on the reaction, and attempting to initiate the reaction with AIBN and no catalyst yielded no products in 12 h of reaction time. These experiments indicate a free radical chain mechanism.

The oxidation of adamantane by this cobalt–acetonitrile species was also studied. Adamantane possesses four tertiary and twelve secondary carbon–hydrogen bonds. The C^2/C^3 ratio, where C^2 is the total of the oxidized products at the secondary position and C^3 is the total of the tertiary alcohol formed, is commonly used as a probe in measuring the selectivity of a reaction being studied. From probability considerations alone, a totally nonselective reaction would lead to a C^2/C^3 ratio of 3. Hydrogen atom abstraction reactions using substituted ferric porphyrin systems result in C^2/C^3 ratios of 0.2.[10,11] Reported nonradical reactions, such as Barton's "Gif" systems,[12] which involve an iron catalyst, have also been probed using this substrate. A C^2/C^3 ratio of 3.7 is observed. Modification of the Gif system has resulted in C^2/C^3 ratios as high as 15.[13] On the basis of these studies, it has been

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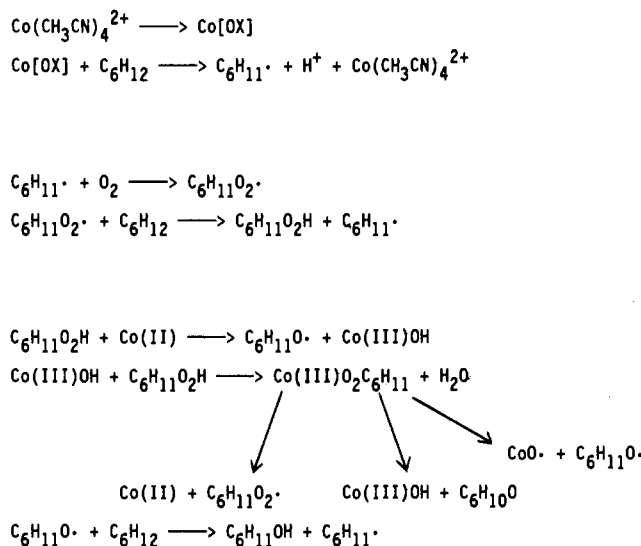
$$Co(CH_3CN)_4{}^{2+} \longrightarrow Co[OX]$$

$$Co[OX] + C_6H_{12} \longrightarrow C_6H_{11}{}^{\cdot} + H^+ + Co(CH_3CN)_4{}^{2+}$$

$$C_6H_{11}{}^{\cdot} + O_2 \longrightarrow C_6H_{11}O_2{}^{\cdot}$$

$$C_6H_{11}O_2{}^{\cdot} + C_6H_{12} \longrightarrow C_6H_{11}O_2H + C_6H_{11}{}^{\cdot}$$

$$C_6H_{11}O_2H + Co(II) \longrightarrow C_6H_{11}O^{\cdot} + Co(III)OH$$

$$Co(III)OH + C_6H_{11}O_2H \longrightarrow Co(III)O_2C_6H_{11} + H_2O$$

$$Co(II) + C_6H_{11}O_2{}^{\cdot} \qquad Co(III)OH + C_6H_{10}O \qquad CoO^{\cdot} + C_6H_{11}O^{\cdot}$$

$$C_6H_{11}O^{\cdot} + C_6H_{12} \longrightarrow C_6H_{11}OH + C_6H_{11}{}^{\cdot}$$

Figure 4. Proposed mechanism for the oxidation of cyclohexane.

proposed that the Gif systems are proceeding via a nonradical mechanism. In a typical oxidation reaction using the cobalt–acetonitrile species, adamantane was oxidized to 1-adamantanol (65%), 2-adamantanol (25%), and 2-adamantanone (10%). This corresponds to $C^2/C^3 = 0.53$, indicative of a radical process.

Our proposed mechanism for the oxidation of cyclohexane is illustrated in Figure 4. It differs slightly from reported mechanisms which include a step found in hydroperoxide decomposition that involves $RO_2{}^{\cdot}$ coupling to form RO_4R, which then decomposes to O_2, ketone, and alcohol. We find the proposed accumulation of $RO_2{}^{\cdot}$ that occurs in pure hydroperoxide or inert solvents unlikely in cyclohexane and base our model on recent studies of metal-catalyzed peroxide decompositions.[14] During the oxidation, a 2-h induction period is observed. The nature of this induction period was investigated to determine whether the reaction is being initiated at low peroxide thresholds or if an oxidized cobalt species reacts with the substrate to initiate the reaction. At the beginning of the reaction 10^{-5} mol of *tert*-butylhydroperoxide was added to determine if peroxides are initiating the reaction. Upon addition of 2×10^{-5} mol of peroxide, no change in the induction period was observed. Another possible explanation of this induction period involves the oxidation of some of the cobalt(II) by O_2 to an oxidized cobalt species, Co[OX], whose exact composition is unknown. To determine if this is the cause of the induction period, the catalyst in acetonitrile, in the absence of the substrate, was pressurized at 75 °C for 2 h, the length of the induction period. After this time, the substrate was added. An induction period for the oxidation reaction was still observed, but it decreased to approximately $^1/_2$ h (see Figure 2). This suggests that the oxidation of the cobalt ion is occurring during the induction period to produce a metal ion species that can react with the substrate forming a cyclohexyl radical. A variety of other reactions involving metals such as Co^{3+} or Fe^{3+} have been reported to generate radical species via electron-transfer processes.[15–17]

Electrochemical experiments demonstrated the feasibility of radical initiation via an electron-transfer process. The cobalt species in acetonitrile resulted in a quasi-reversible wave in the cyclic voltamogram at a potential of 1.9 V vs NHE. This oxidation wave is a result of the oxidation of Co^{2+} to a higher oxidized species (Co[OX]). The electrochemistry of the commercial catalyst,
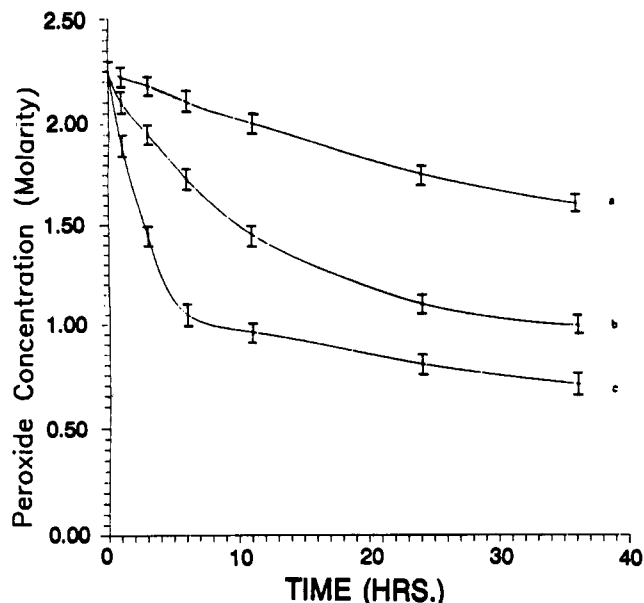
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Figure 5. Decomposition of *t*-BuOOH at 75 °C and 3 atm: (a) no catalyst; (b) [Co(BPI)$_2$]; (c) [Co(NCCH$_3$)$_4$](PF$_6$)$_2$.

Co(oct)$_2$, which showed no activity under our reaction conditions, resulted in an irreversible wave at 1.2 V vs NHE. This potential indicates that this oxidized cobalt complex would be less efficient at initiating the reaction and explains the lack of activity of this catalyst under mild reaction conditions. Under the reaction conditions, a solution of anhydrous CoCl$_2$ in acetonitrile showed one-third of the activity of [Co(NCCH$_3$)$_4$](PF$_6$)$_2$. Cyclic voltammetry of CoCl$_2$ in acetonitrile resulted in an oxidation wave at 1.6 V vs NHE. A small wave due to the oxidation of free chloride is observed at lower potentials, 1.3 V. The loss of activity which occurs with the formation of water, as previously discussed, has been supported by electrochemical studies. The addition of water to a solution containing the cobalt ion species in acetonitrile resulted in a decrease in the oxidation potential for the cobalt from 1.9 V to a value of 1.2 V vs NHE. The hydrated cobalt species is not a potent enough oxidant to remove an electron from cyclohexane, resulting in a loss of activity. The potentials reported here all correspond to irreversible cyclic voltammograms and at best should be used to illustrate trends that are consistent with the observed reaction chemistry. These factors and solvation negate direct comparison of these potentials with the reported reversible potential for O_2 reduction to deduce the feasibility of cobalt oxidation by O_2. Furthermore, reaction conditions differ from those employed in the electrochemistry.

During the 2-h induction period, the buildup of the oxidized cobalt occurs until its concentration is high enough to initiate the reaction by reacting with cyclohexane to generate the cyclohexyl radical. The alkyl radical generated in the first step reacts readily with dioxygen forming the alkyl hydroperoxy radical. This radical then reacts with cyclohexane generating another alkyl radical and cyclohexyl hydroperoxide. Once formed, the hydroperoxide then undergoes a Haber–Weiss decomposition mechanism[13,18] forming the observed products, cyclohexanol and cyclohexanone.

The effectiveness of the cobalt–acetonitrile species in catalyzing the decomposition of peroxides was studied using *t*-BuOOH as a model peroxide under the oxidation reaction conditions. The concentration of peroxide was measured using an iodometric titration procedure.[19] As illustrated in Figure 5, [Co(NC-CH$_3$)$_4$](PF$_6$)$_2$ decomposes *t*-BuOOH more efficiently than that reported for the efficient CoBPI complexes (BPI = 1,3-bis(2-pyridylimino)isoindoline).[14,15] With both catalysts, water produced during the decomposition solvates the cobalt and, after approx-

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imately 8 h of reaction time, leads to the loss of catalytic activity for peroxide decomposition.

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Comparative ¹H NMR Study of Ferric Low-Spin Cytochrome c Peroxidase and Horseradish Peroxidase

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Proton NMR spectra of the cyanide complexes of cytochrome c peroxidase from baker's yeast (CcP) and recombinant protein expressed in *Escherichia coli* (MKT-IGCcP) have been recorded in water. The paramagnetically shifted exchangeable proton signals have been assigned through NOE and NOESY experiments. NOESY and COSY experiments in D₂O and H₂O buffers have allowed us to extend the assignments to heme substituents and other residues. Comparison is made with the analogous spectrum of the cyanide adduct of horseradish peroxidase (HRP) (Thanabal, V.; de Ropp, J. S.; La Mar, G. N. *J. Am. Chem. Soc.* **1988**, *110*, 3027). HRP–CN⁻ and CcP–CN⁻ systems are highly analogous as shown by the similarity of the pseudocontact shifts of the distal histidine protons and by the protonation status of the proximal and distal histidine in both systems. A more extensive exchangeable proton connectivity network is observed in CcP–CN⁻. Small but significant structural differences have been identified in the orientation of the proximal histidyl residues of HRP–CN⁻ and CcP–CN⁻ and in the interresidue distances in the distal heme pocket of CcP–CN⁻ and MKT-IGCcP–CN⁻ as a function of the identity of the residue present at position 53.

Peroxidases are a class of heme proteins that react with H₂O₂ or alkyl peroxides to perform a variety of oxidation reactions.[1] Among peroxidases, cytochrome c peroxidase (CcP) and horseradish peroxidase (HRP) have been studied in greatest detail by a variety of physical and spectroscopic techniques.[1] In the resting state, the heme iron of most peroxidases is high-spin Fe(III), though the HRP heme iron has been proposed to possess a spin admixture with $S = {}^3/_2$.[2] The fifth coordination position of the Fe(III) ion in peroxidases is occupied by the nitrogen atom of a histidyl residue (the proximal histidine)[3] in all known members of this family. Characterization of the sixth coordination position of peroxidases in the resting state has been the subject of intense study.[3d,4]

While HRP in the resting state is thought to be five-coordinate,[4c] a consensus concerning the coordination environment of resting state CcP has been more difficult to achieve.[4] Refined structural models have been reported for the ferric ("resting state") enzyme[3b] and for the CN⁻ adduct.[5] The two structures are highly similar except for the movement of the iron atom toward the heme plane and the removal of one water molecule from the distal side of the heme upon CN⁻ binding. Several critical amino acid residues and water molecules occurring on both sides of the heme prosthetic group in the CN⁻-bound form of the protein are illustrated in Figure 1.

Reaction of peroxidases with H₂O₂ produces the active oxidizing form of the protein, the electronic structure of which varies between peroxidases. Historically, this form of CcP is referred to as compound ES, and the corresponding form of HRP is referred to as compound I.[1] Despite the close similarity in heme iron coordination geometries of HRP compound I and CcP compound ES,[6] their electronic structures are substantially different. Both derivatives are oxidized by 2 equiv above the resting enzyme and possess a ferryl heme iron (Fe^{IV}=O); however, they differ in that compound ES possesses a radical centered on Trp-191[7] while HRP possesses a porphyrin π-cation-radical center.[8]

Both resting enzymes bind cyanide as a sixth ligand to produce a species with low-spin Fe(III); the three-dimensional structure

of CcP–CN⁻ has been reported recently (Figure 1).[5] Binding of cyanide displaces the water molecule that is located closest to the iron atom and results in a rearrangement of the hydrogen-bonding pattern of the remaining two water molecules;[5] both of the remaining water molecules form hydrogen bonds with the nitrogen atom of the cyanide group. One of these water molecules also forms a hydrogen bond with Arg-48. Crystallographic analysis also reveals that Arg-48, Trp-51, and His-52 are all within hydrogen-bonding distance of the cyanide nitrogen atom. As hydrogen bond formation with the bound cyanide anion requires hydrogen atom donation by the putative hydrogen bonding partner, Arg-48 and Trp-51 are both capable of hydrogen bonding to this group as both groups meet this requirement. However, it is not currently apparent whether or not His-52 meets this criterion because the protonation status of this residue cannot be determined from the current structural model of the protein.[5]

Assignments of the ¹H NMR spectrum of HRP–CN⁻ has been one of the most elegant applications of ¹H NMR spectroscopy

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