ADSORPTION AND ELECTROCHEMISTRY OF BIS-1,10-PHENANTHROLINE COMPLEXES OF COPPER (I,II) AND FUNGAL LACCASE A

Thesis by

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Dedicated to my parents
on the occasion of their sixtieth birthdays

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ABSTRACT

This thesis is an outcome of the efforts directed toward the theme "Electrocatalysis of dioxygen reduction by copper-containing molecules adsorbed on graphite electrodes."

In the first part, an electrochemical measurement of the rate of electron exchange between $Cu(phen)_2^+$ adsorbed on graphite and $Cu(phen)_2^{++}$ in solution is described. The rate constant was estimated to be ca. 10^5 M^{-1} s^{-1} by using rotating disk voltammetry. The origin of the previously reported but widely discrepant values (5 x 10^7 M^{-1} s^{-1} and 50 M^{-1} s^{-1}) in the literature is discussed, and a new estimate of ca. 10^4 M^{-1} s^{-1} is obtained by applying Marcus theory in a form that allows explicitly for differences in reorganization energy between the oxidized and reduced halves of the reacting redox couples. The electrocatalytic studies of dioxygen reduction by $Cu(phen)_2^+$ could not be done due to the low signal-to-noise ratio.

In the second part, the bioelectrocatalytic reduction of molecular oxygen by reduced fungal laccase A immobilized on pyrolytic graphite is described. The reduction produces exclusively water in the potential range studied (> 0 V vs. SSCE). The catalytic activity reaches its maximum at pH 3~4, and the lower limit on rate constant for the reduction in this pH range was estimated to be 1.5 x 10^4 M^{-1} s^{-1}.

Three appendices describe (i) potential step hydrodynamic chronocoulometry, (ii) a new method to determine the self-exchange rate constant for the redox couple in which the coordination numbers depend on the oxidation states, and (iii) a novel electrochemical method of detecting H_2O_2.

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CHAPTER I

INTRODUCTION

The energy problems faced internationally have prompted increased interest in electrocatalysis (1-10). Major areas of current research include O_2 evolution (2) and reduction (3), H_2 oxidation (4) and evolution (5), Cl_2 generation (6), and electrooxidation of organic compounds (7). Several reviews (8-10) have been published describing recent developments in the field. This thesis concerns only O_2 reduction at modified electrodes, in particular with copper-containing molecules as modifying agents or electrocatalysts.

With the advent of modified electrodes, which were first demonstrated by Lane and Hubbard (11), the catalysis of electroreduction of molecular oxygen has progressed remarkably. The research direction has been modified to the use of rather cheap, flexible catalysts adsorbed or otherwise anchored on the electrode surface (12-15) instead of the classical method of using expensive noble metals (16). Noble metal electrocatalysis, in addition to the economic limitations, has its own disadvantage; platinum, the best catalyst known for the direct reduction of molecular oxygen to water, needs a significant overvoltage which limits the efficiency of the fuel cell (17). A fuel cell is a device for the direct conversion of chemical energy to electrical energy, at whose cathode the reduction of molecular oxygen takes place. With modified electrodes, however, it is anticipated that virtually any potential catalyst can be used for the catalytic redox reactions after being directly attached on electrode surfaces via simple adsorption (18) or covalent anchoring (19) or indirectly via polymer films on electrode surfaces which have incorporated sites (20, 21). The methodology used in this study is based on the fact that aromatic compounds (18) or other molecules containing aromatic centers (22) adsorb on graphite electrodes.

Many different types of materials have been examined as potential electrocatalysts for dioxygen reduction (8-10, 12-16). Most of them are metalloporphyrins or phthalocyanines with transition metal centers, especially cobalt or iron. Elegant catalysis was obtained with the use of two transition metal catalysts acting in series to accomplish the overall four-electron reduction of molecular oxygen to water (14) or dicobalt face-to-face porphyrin with four-atom linkage between the porphyrin rings for the direct reduction of molecular oxygen to water (13). The highest potential achieved for the four electron reduction of molecular oxygen to water is +0.42V (VS. SCE) or +0.66V (VS. NHE) at pH 1 (13). This is still much lower than the thermodynamic potential for the reaction. Thermodynamic constraints are governed by equation (1).

$$O_2 + 4H^+ + 4e^- \rightleftarrows 2H_2O \quad E = 1.23\ V \qquad (1)$$

(The potential is the reversible thermodynamic potential in 1M acid versus a normal hydrogen electrode (23).) One of the current research efforts in this respect is to shift the catalytic potential as close as possible to the thermodynamic potential with a reasonable rate.

Potential electrocatalysts to be studied would be copper-containing molecules (24-29). They have attracted chemists' attention over decades because copper exists as a cofactor, in spectroscopically unusual forms, in biological systems (24), and, in several cases, copper-containing proteins have been shown to be effective for the reduction of molecular oxygen (25-27). In this context, it would be very interesting to know whether simple copper complexes and/or biological copper-containing molecules will catalyze the reduction of molecular oxygen on electrode surfaces, and whether they have any advantages over the electrocatalysts known thus far.

In the first endeavors, $Cu(phen)_2^+$ was chosen. It is a simple copper complex with aromatic anchors which through homogeneous studies (28) is known to be an effective reagent for the reduction of molecular oxygen and which is claimed to be a model for a copper protein (29). In Chapter II (30), the adsorption behavior of the molecule is described with the emphasis on the electron exchange between $Cu(phen)_2^+$ on an electrode surface and $Cu(phen)_2^{++}$ in solution. Possible implications on dioxygen reduction at the surface of graphite electrode are also presented.

In Chapter III (31), bioelectrocatalytic reduction of molecular oxygen by reduced fungal laccase A is described. This enzyme, which is a four-copper protein, was immobilized on a pyrolytic graphite electrode by adsorption. The products of reaction, the electrode potential where the reaction proceeds and the mechanistic role of protons were among the topics examined. Features of the catalytic reaction which yield insights into the mechanism are also discussed.

In Appendix I, a new electrochemical technique of potential step hydrodynamic chronocoulometry is described. This technique was applied to the measurement of the Cu(I) accumulated on the surface of a rotating disk electrode in Chapter II. In Appendix II (32), rate constants for electron self-exchange between the 1,10-phenanthroline and 2,2'-bipyridine complexes of Cu(I) and Cu(II) were recalculated from existing experimental data without making the common assumption that the contribution of one redox couple to the reorganization energy of its cross-reactions is independent of the identity of the second redox couple engaged in the cross-reaction. In Appendix III, a novel electrochemical methodology to detect H_2O_2 is described. This method was employed to analyze the products in the electroreduction of molecular oxygen by reduced fungal laccase A in Chapter III.

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CHAPTER II

ELECTRON EXCHANGE BETWEEN $Cu(phen)_2^{+}$ ADSORBED ON GRAPHITE AND $Cu(phen)_2^{2+}$ IN SOLUTION

INTRODUCTION

One of the simplest methods for attaching metal complexes to the surfaces of graphite electrodes takes advantage of the high affinity for graphite of molecules having multiple aromatic centers (1). Coordination of polypyridine ligands to metal centers creates complexes that show affinity for graphite surfaces, and we have exploited this fact to bind the bis-1,10-phenanthroline (phen) complexes of Cu(II) and Cu(I) to pyrolytic graphite electrodes from aqueous media. In addition, the Cu(I) complex appears to oligomerize in solution. This enhances its tendency to accumulate at the graphite-solution interface and causes large quantities of the complex to deposit on the electrode surface. The rate of electron transfer between the deposited $Cu(phen)_2^{+}$ complex and $Cu(phen)_2^{2+}$ ions dissolved in solution was measured by means of rotating disk electrode voltammetry (2,3). This electron transfer rate is of interest in assessing the potential of deposited $Cu(phen)_2^{+}$ as an electrocatalyst for the reduction of dioxygen (4). In addition, there is disagreement in the literature regarding the rate of homogeneous electron exchange between $Cu(phen)_2^+$ and $Cu(phen)_2^{2+}$ (5), and we expected that an electrochemical estimate of the same rate could help to resolve the disagreement.

EXPERIMENTAL

Materials. $Cu(phen)_2Cl_2\cdot 3H_2O$ was prepared by dissolving a slight excess of 1,10-phenanthroline monohydrate (J. T. Baker Co). in warm ethanol and adding it to a solution of $CuCl_2\cdot 2H_2O$ dissolved in an equal volume of warm water. The crystals that formed after the solution stood overnight were collected, washed with ethanol and dried under vacuum. Elemental analysis: C = 53.30%; H = 4.03%; Cl = 13.03%; calculated for the stated formula C = 52.51%; H = 4.04%; Cl = 12.92%. $Cu(phen)_2(CF_3SO_3)_2(H_2O)_1$ was prepared similarly using the $Cu(CF_3SO_3)_2$ stock solution, instead of $CuCl_2$, which had been obtained from heating the mixture of excess cupric carbonate and trifuoromethanesulfonic acid (Minnesota Mining and Manufacturing Co.) to a boil and filtering it. Elemental analysis: Cu = 8.75%; C = 42.85%; H = 2.55%; N = 7.68%; S = 8.90%; calculated for the stated formula Cu = 8.59%; C = 42.19%; H = 2.46%; N = 7.57%; S = 8.66%.

All other chemicals were reagent grade and were used as received. Solutions were prepared from laboratory deionized water that was passed through a purification train (Barnsted Nanopure) before use. Solutions were deoxygenated with prepurified argon and buffered at pH 5.6 with 0.02 M acetate buffer. Electrodes were prepared from basal plane pyrolytic graphite (Union Carbine Co., Chicago, Ill.) and were mounted as stationary or rotating disk electrodes as previously described (3). The electrodes had an exposed area of 0.17 cm^2. The reference electrode was a saturated calomel electrode (SCE) except when the solvent was dimethyl formamide where a silver-silver chloride reference electrode was used. Quoted potentials are with respect to the SCE. Experiments were conducted at the laboratory temperature, 22 ± 2°C.

Apparatus and Techniques. Cyclic voltammetry and rotating disk voltammetry were conducted with apparatus and procedures described previously (3,4). Absorption spectra were recorded with a Hewlett-Packard Model 8450 spectrophotometer. Controlled potential reductions of $Cu(phen)_2^{2+}$ were conducted inside a glove bag (to exclude dioxygen rigorously) with a pyrolytic graphite plate as working electrode. Chronocoulometric measurements (7) were performed with a computer-based apparatus similar to one previously described (8).

The application of chronocoulometry to the measurement of adsorption at rotating disk electrodes has not previously been described. The following procedure was employed: the quantity of $Cu(phen)_2^+$ deposited on rotating graphite disk electrodes by the reduction of $Cu(phen)_2^{2+}$ was measured by stepping the potential of the continuously rotating electrode from the value where the reductive deposition of $Cu(phen)_2^+$ was rapidly re-oxidized to $Cu(phen)_2^{2+}$. The total charge consumed during the oxidation was monitored as a function of time. The only significant sources of $Cu(phen)_2{}^+$ are the deposit itself and the "Levich layer" at the electrode surface (9) because the rest of the electrogenerated $Cu(phen)_2{}^+$ is stirred into the large volume of the solution of $Cu(phen)_2{}^{2+}$ and is thus diluted to a negligibly small concentration. As a result, the charge-time transient during the oxidation step consists of a rapid rise followed by an essentially flat plateau that can be linearily extrapolated to zero time, $t = 0$, to obtain, from Faraday's Law, an estimate of the quantity of $Cu(phen)_2{}^+$ that had been deposited. First there must be subtracted from the charge at $t = 0$ the charge measured in a blank experiment in the absence of the $Cu(phen)_2{}^{2+}$ plus the charge associated with the $Cu(phen)_2{}^+$ in the Levich layer. The contribution from the latter source was estimated as $\frac{FC^b\delta}{2}$ where F is the Faraday, C^b is the concentration of $Cu(phen)_2{}^{2+}$ in the bulk solution and δ is the thickness of the Levich layer (cm). In aqueous solutions for a species with diffusion coefficient $5 \times 10^{-6}\ cm^2 s^{-1}$, $\delta = 1.28 \times 10^{-2}\ \omega^{-\frac{1}{2}}$ (cm) where ω is the angular frequency of rotation of the electrode. At a rotation rate of 1000 rpm and a concentration of 1 mM the maximum contribution from the Levich layer amounts to 6×10^{-10} moles cm^{-2} and it becomes proportionally smaller at lower concentrations or higher rotation rate. The reliability of this technique was tested with solutions of $Ru(NH_3)_6{}^{3+}$ which is not adsorbed on graphite (see

Appendix I). A sharply stepped charge-time transient resulted when the potential was stepped from a value where $Ru(NH_3)_6^{3+}$ was being reduced to $Ru(NH_3)_6^{2+}$ to a value where the opposite was true and the charge on the plateau of the transient closely matched the sum of the charge measured in the blank experiment plus the contribution from the Levich layer as calculated from the formula given here.

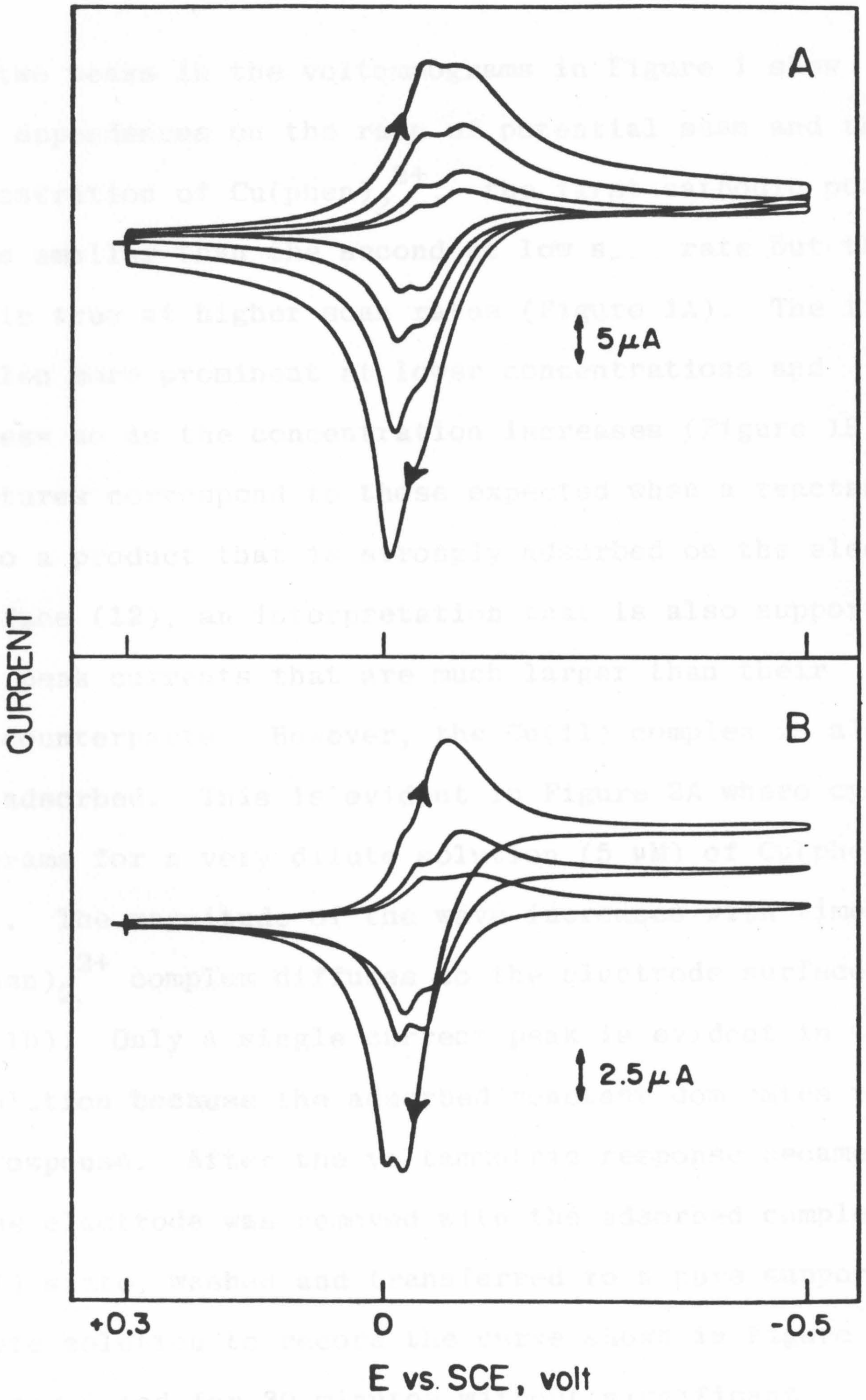
RESULTS

Cyclic Voltammetry. The cyclic voltammetry of $Cu(phen)_2^{2+}$ was studied in chloride rather than perchlorate supporting electrolytes to increase the solubility of the $Cu(phen)_2^{+}$ produced at the electrode. The solution was buffered at pH 5.6 to assure the coordinative stability of the complexes. The voltammograms, shown in Figure 1, contain two current peaks at potentials where $Cu(phen)_2^{2+}$ is reduced to $Cu(phen)_2^{+}$. A second irreversible wave corresponding to the reduction of $Cu(phen)_2^{+}$ to Cu appears at potentials more negative than -1 volt and was not examined in this study. The reported dissociation constant for $Cu(phen)_2^{+}$, $K_d = 1.6 \times 10^{-16}\ M^2$ (10), combined with the standard potential of the $Cu^{+}aq/Cu$ couple, $E^o = 0.28$ volt vs SCE (11), gives a calculated potential of -0.65 volt for the half-reaction $Cu(phen)_2^{+} + e = Cu + 2\ phen$ at (phen) = 1 M. In the absence of excess phen a 1 mM solution of $Cu(phen)_2^{+}$ would therefore be expected to undergo reversible reduction to Cu near -0.3 volt vs. SCE.

Figure 1. Cyclic voltammograms of Cu(phen)$_2^{2+}$ at a pyrolytic graphite electrode. Supporting electrolyte: 0.1 M NaCl buffered at pH 5.6 with acetate (0.02 M).

A. (Cu(phen)$_2^{2+}$): 0.4 mM; scan rates: 10, 20, 50, 100 mV s^{-1}.

B. Scan rate: 10 mV s^{-1}, (Cu(phen)$_2^{2+}$): 2, 4, 8 x 10^{-4} M.

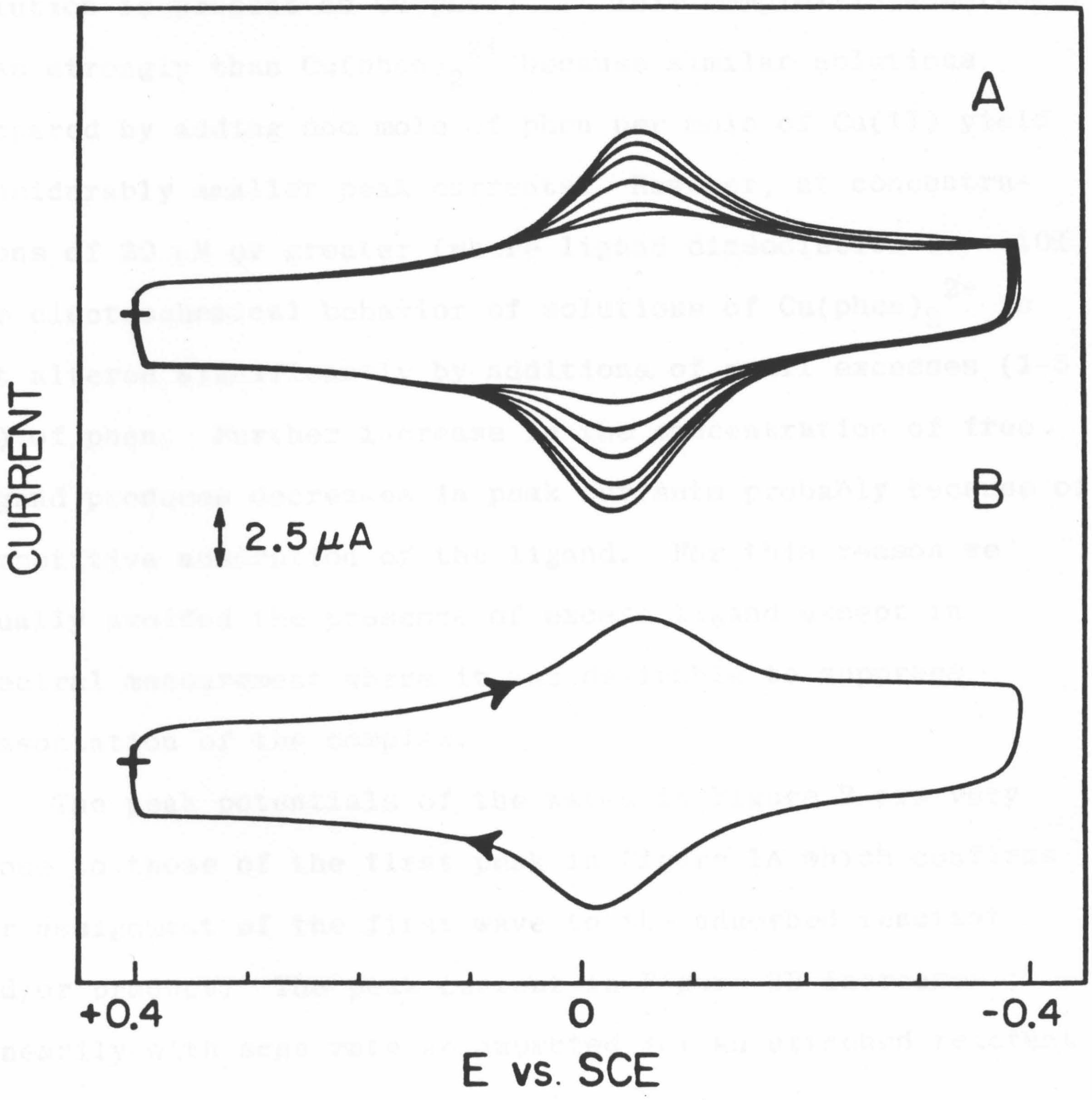

A
5 μA
CURRENT
B
2.5 μA
+0.3
0
-0.5
E vs. SCE, volt

The much more negative potentials that are required before the reaction actually occurs show it to be highly irreversible.

The two peaks in the voltammograms in Figure 1 show different dependences on the rate of potential scan and the bulk concentration of $Cu(phen)_2^{2+}$: the first cathodic peak current is smaller than the second at low scan rate but the converse is true at higher scan rates (Figure 1A). The first peak is also more prominent at lower concentrations and becomes less so as the concentration increases (Figure 1B). These features correspond to those expected when a reactant is reduced to a product that is strongly adsorbed on the electrode surface (12), an interpretation that is also supported by anodic peak currents that are much larger than their cathodic counterparts. However, the Cu(II) complex is also strongly adsorbed. This is evident in Figure 2A where cyclic voltammograms for a very dilute solution (5 μM) of $Cu(phen)_2^{2+}$ are shown. The magnitude of the wave increases with time as the $Cu(phen)_2^{2+}$ complex diffuses to the electrode surface and adsorbs (1b). Only a single current peak is evident in this dilute solution because the adsorbed reactant dominates the overall response. After the voltammetric response became steady the electrode was removed with the adsorbed complex in the Cu(II) state, washed and transferred to a pure supporting electrolyte solution to record the curve shown in Figure 2B. The wave persisted for 30 minutes without significant

Figure 2. A. Successive cyclic voltammograms recorded in a 5 μM solution of $Cu(phen)_2^{2+}$. The potential was maintained at 0.4 volt between scans. The curves shown were recorded 0.2, 1, 2, 5, 10 and 20 min. after the freshly cleaved electrode was immersed in the solution.

B. After the electrode was removed, washed and transferred to pure supporting electrolyte solution. (0.1 M NaCl; pH 5.6). Scan rate: 200 mV s^{-1}.


A
B
CURRENT
2.5 μA
+0.4
0
-0.4
E vs. SCE

diminishment showing that both the Cu(I) and Cu(II) complexes are adsorbed irreversibly on the electrode surface.

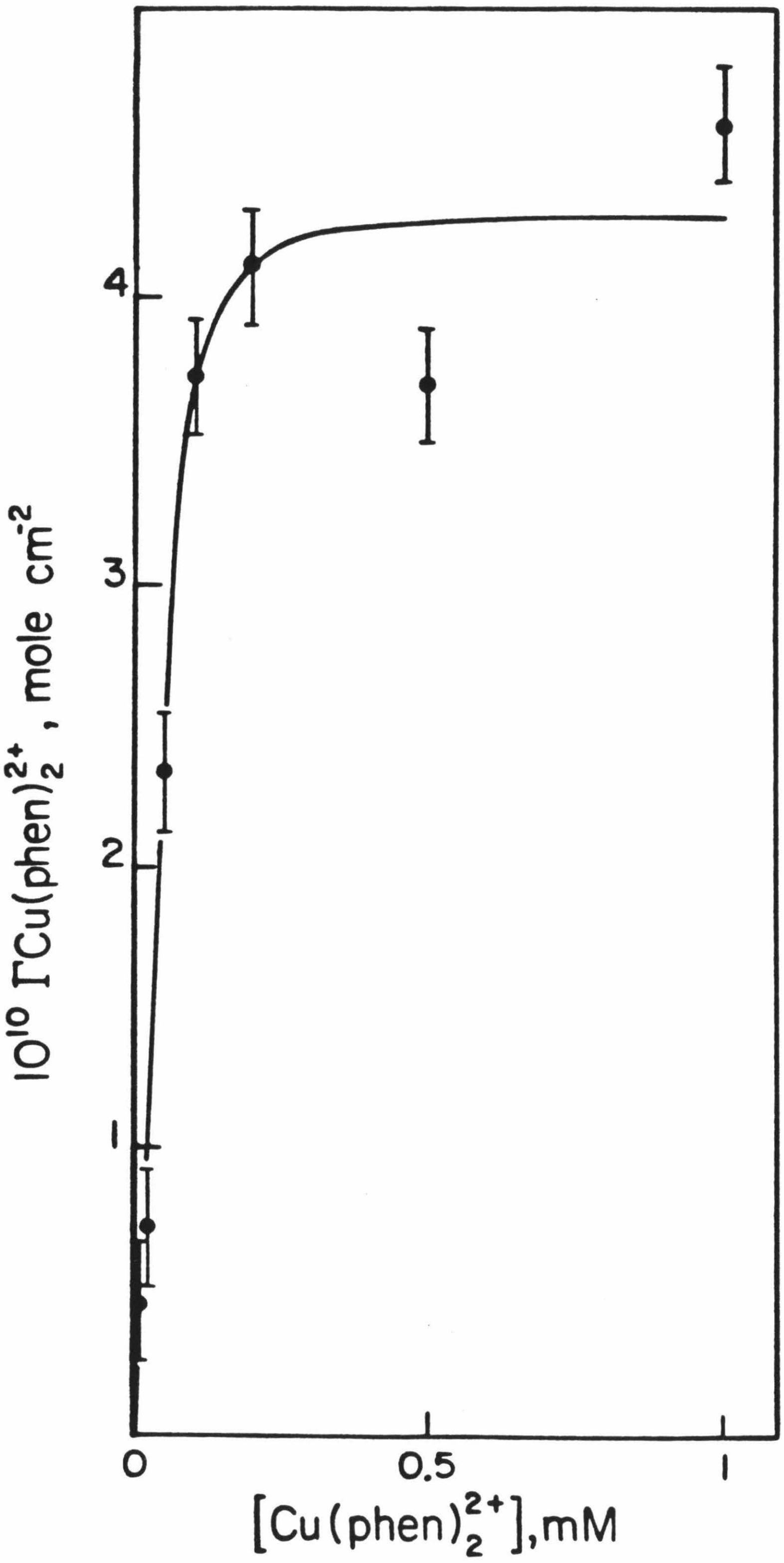
The first ligand dissociation constant of $Cu(phen)_2^{2+}$ is $10^{-6.6}$ M (10) so that about 20% of the complex in a 5 μM solution is present as $Cu(phen)^{2+}$. This complex is adsorbed less strongly than $Cu(phen)_2^{2+}$ because similar solutions prepared by adding one mole of phen per mole of Cu(II) yield considerably smaller peak currents. However, at concentrations of 20 μM or greater (where ligand dissociation is < 10%) the electrochemical behavior of solutions of $Cu(phen)_2^{2+}$ is not altered significantly by additions of small excesses (1-5 μM) of phen. Further increase in the concentration of free ligand produces decreases in peak currents probably because of competitive adsorption of the ligand. For this reason we usually avoided the presence of excess ligand except in spectral measurement where it was desirable to suppress dissociation of the complex.

The peak potentials of the waves in Figure 2 are very close to those of the first peak in Figure 1A which confirms our assignment of the first wave to the adsorbed reactant and/or product. The peak current in Figure 2B increases linearily with scan rate as expected for an attached reactant (13).

Measurement of Adsorption of $Cu(phen)_2^{2+}$ by Chronocoulemtry. The extent of the adsorption of $Cu(phen)_2^{2+}$ was estimated from single step chronocoulometric measurements (7) in which the potential of the electrode was stepped from +0.3 to -0.4 volt. The intercepts of the resulting charge-(time)$^{\frac{1}{2}}$ plots were corrected for background and double layer charging effects by subtracting the charge obtained when the same potential step was applied in the absence of $Cu(phen)_2^{2+}$. The background corrections were relatively large with pyrolytic graphite electrodes, ca. 40 μC cm^{-2}, and experiments were repeated with several electrodes to obtain representative average values of the adsorption. The dependence of the adsorption of the complex on its bulk concentration is shown in Figure 3. Full coverage is apparently reached at concentrations of ca. 0.1 mM. The maximum adsorption of ca. 4×10^{-10} mole cm^{-2} corresponds to a monolayer of close-packed $Cu(phen)_2^{2+}$ taking the diameter of the complex as 14 Å (5a, 14) and the microscopic area of the electrode as ca. three times larger than its geometric area.

<u>Spectral Properties of Solutions of $Cu(phen)_2^{+}$.</u> The few previous spectral studies of solutions of $Cu(phen)_2^{+}$ (5a, 15, 16) contain evidence of concentration dependent speciation in the form of shifting values of λ_{max} and molar absorbances. For example, the color of aqueous solutions of the complex changes from purple to yellow as the concentration of complex is decreased from ca. 1 mM to 50 μM (15). To examine this phenomenon in our 0.1 M NaCl supporting electrolyte a series of solutions of $Cu(phen)_2^{+}$ were prepared by electrolytic reduction (at -0.5 volt) of $Cu(phen)_2^{2+}$ solutions that also

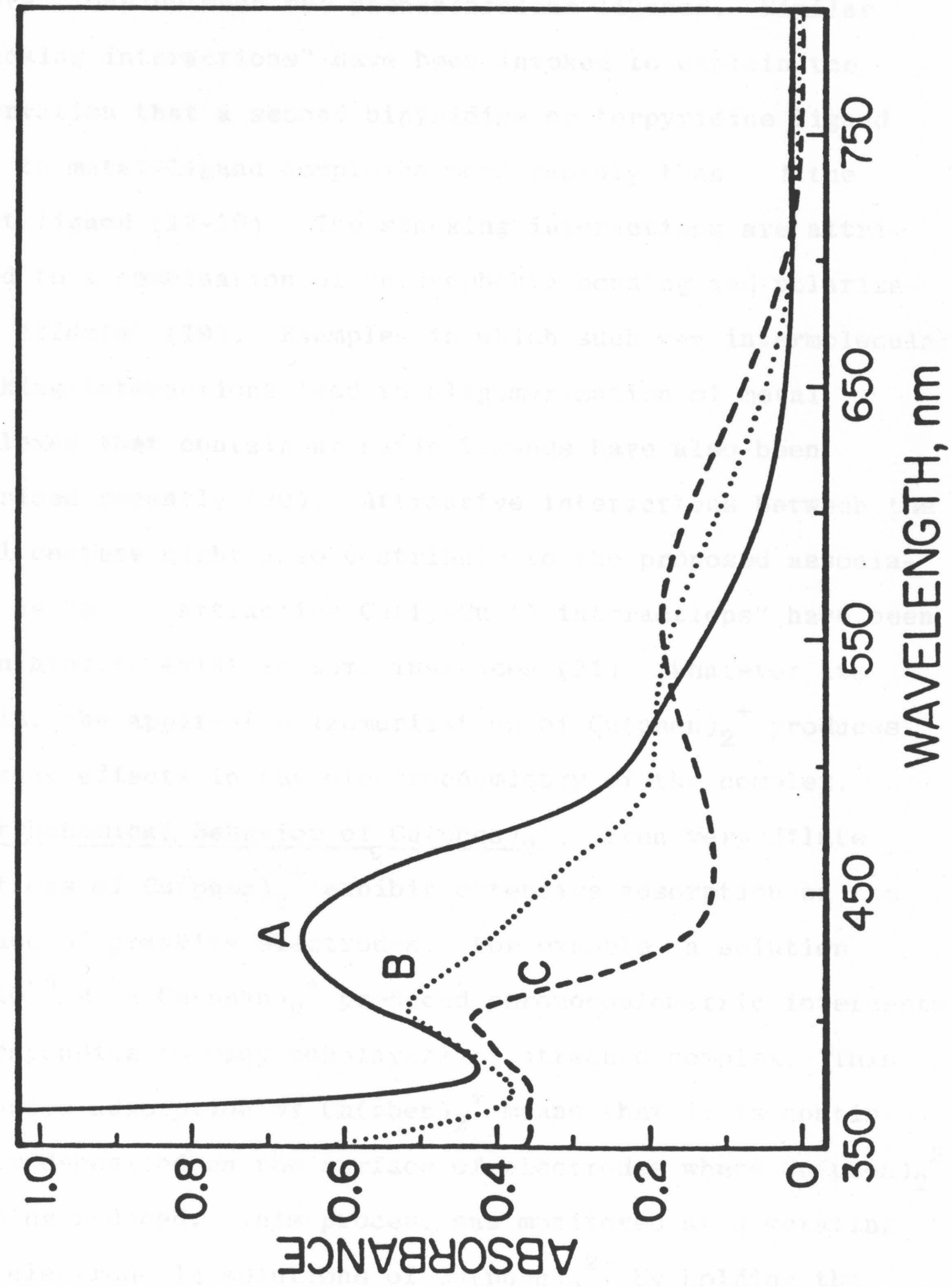
Figure 3. Adsorption of $Cu(phen)_2^{2+}$ at pyrolytic graphite electrodes. Supporting electrolyte: 0.1 M NaCl (pH 5.6).


$10^{10}\ \Gamma Cu(phen)_2^{2+}$, mole cm^{-2}
1
2
3
4
0
0.5
1
$[Cu(phen)_2^{2+}]$, mM

contained 2 mM excess ligand to suppress dissociation of the complex. The electrolysis was carried out inside a controlled atmosphere box where dioxygen was rigorously excluded. After completion of the electrolysis, portions of the solution were transferred to cuvettes, sealed and spectra recorded. The three representative spectra shown in Figure 4 were recorded with different cuvettes chosen so that the product of the optical path length and the concentration of complex remained constant. At lower concentrations the spectrum is dominated by the band at 434 nm. As the concentration increases two new bands appear, first at 402 nm and later at 554 nm, as the intensity of the band at 434 nm diminishes. The same qualitative behavior was also obtained when solutions containing only $Cu(phen)_2(CF_3SO_3)_2$ were treated with ascorbic acid to obtain the $Cu(phen)_2^+$ complex in the absence of chloride so that the behavior does not appear to be a function of the anion present.

The behavior observed could result from oligomerization of the $Cu(phen)_2^+$ complexes as their concentration is increased. The spectra of dilute solutions (< ca. 15 μM) correspond to a molar absorbance, ε_{434}, of 6700 M^{-1} cm^{-1}. This is larger than the single previous reported value (16), ε_{410} = 4100 M^{-1} cm^{-1}, but the concentration dependence of the spectra in Figure 4 reveals the origin of the apparent discrepancy: the molar absorbance between 400 and 500 nm is a strong function of the concentration of Cu(I). The

Figure 4. Representative electronic absorption spectra of solutions of $Cu(phen)_2^+$ that exhibit a concentration dependence. Supporting electrolyte: 0.1 M NaCl (pH 5.6 acetate buffer). ($Cu(phen)_2^+$): A 0.01 mM; B 0.10 mM; C 1.0 mM. Optical path length of cuvette: A 10 cm; B 1 cm; C 0.1 cm.

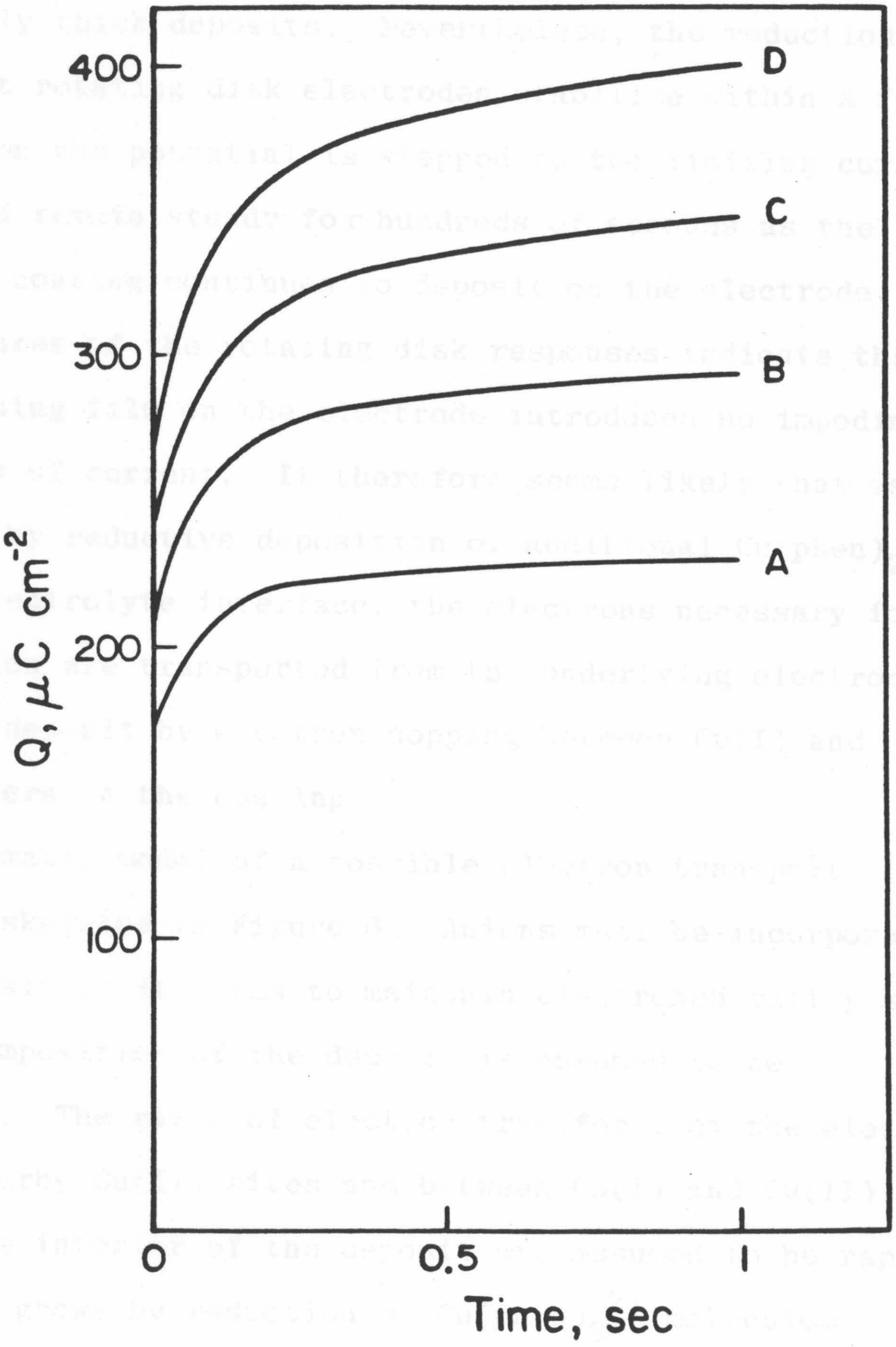

ABSORBANCE
1.0
0.8
0.6
0.4
0.2
0
350
450
550
650
750
WAVELENGTH, nm
A
B
C

concentration dependence of the spectra may result from association of the Cu(I) complexes because of attractive interactions between the phenanthroline ligands. Similar "stacking interactions" have been invoked to explain the observation that a second bipyridine or terpyridine ligand adds to metal-ligand complexes more rapidly than did the first ligand (17-19). The stacking interactions are attributed to a combination of "hydrophobic bonding and polarization effects" (19). Examples in which such π-π intermolecular stacking interactions lead to oligomerization of metal complexes that contain aromatic ligands have also been described recently (20). Attractive interactions between the metal centers might also contribute to the proposed association as "soft, attractive Cu(I)-Cu(I) interactions" have been calculated to exist in some instances (21). Whatever its origin, the apparent oligomerization of $Cu(phen)_2^+$ produces striking effects in the electrochemistry of the complex.

<u>Electrochemical Behavior of $Cu(phen)_2^+$</u>. Even very dilute solutions of $Cu(phen)_2^+$ exhibit extensive adsorption on the surface of graphite electrodes. For example, a solution 5×10^{-5} M in $Cu(phen)_2^+$ produced chronocoulometric intercepts corresponding to many monolayers of attached complex. This extensive adsorption of $Cu(phen)_2^+$ means that it is continuously deposited on the surface of electrodes where $Cu(phen)_2^{2+}$ is being reduced. This process was monitored at a rotating disk electrode in solutions of $Cu(phen)_2^{2+}$ by holding the

electrode potential at -0.5 volt for various times and then stepping the potential to +0.8 volt where the $Cu(phen)_2^+$ that had been deposited on the electrode was very rapidly oxidized. The electrogenerated $Cu(phen)_2^+$ that did not remain on the surface was stirred into the bulk of the solution by the rotating electrode and contributed negligibly to the oxidation current. The charge-time responses that resulted when the electrode was stepped to +0.8 volt were recorded and utilized to estimate the quantity of $Cu(phen)_2^+$ that had been deposited. Figure 5 contains a set of charge-time curves obtained in this way. The curves become relatively flat at times greater than ca. 0.5 seconds although, with thicker coatings, charge continues to accumulate at a low rate for longer times. This may result from $Cu(phen)_2^+$ in the outer portions of the coating disengaging from the electrode surface when the intervening complex is oxidized to $Cu(phen)_2^{2+}$ which is adsorbed much less strongly. Any $Cu(phen)_2^+$ that is detached from the surface would have to diffuse back to the electrode to be oxidized. In any case, the value of charge accumulated after one second (corrected for background and Levich layer contributions as explained in the Experimental section) was used to estimate the quantity of $Cu(phen)_2^+$ deposited on the electrode during the time it was held at -0.5 volt. The quantities of $Cu(phen)_2^+$ that can be deposited on the electrode in a state adherent enough to withstand the stirring resulting from the rotation of the

Figure 5. Chronocoulometric charge-time data at a rotating graphite disk electrode in a 1 mM solution of cu(phen)$_2^{2+}$. The electrode potential was held at -0.5 V for time τ and then stepped to +0.8 volt where the charge-time responses were recorded. Supporting electrolyte: 0.1 M NaCl (pH 5.6); electrode rotation rate: 1600 rpm; values of τ were A 5 s; B 50 s; C 100 s; D 200 s. Plotted charges are the total measured charge less the sum of the charge consumed when the experiment was repeated in the absence of Cu(phen)$_2^{2+}$ and the charge corresponding to the Cu(phen)$_2^{+}$ in the Levich layer (see Experimental).

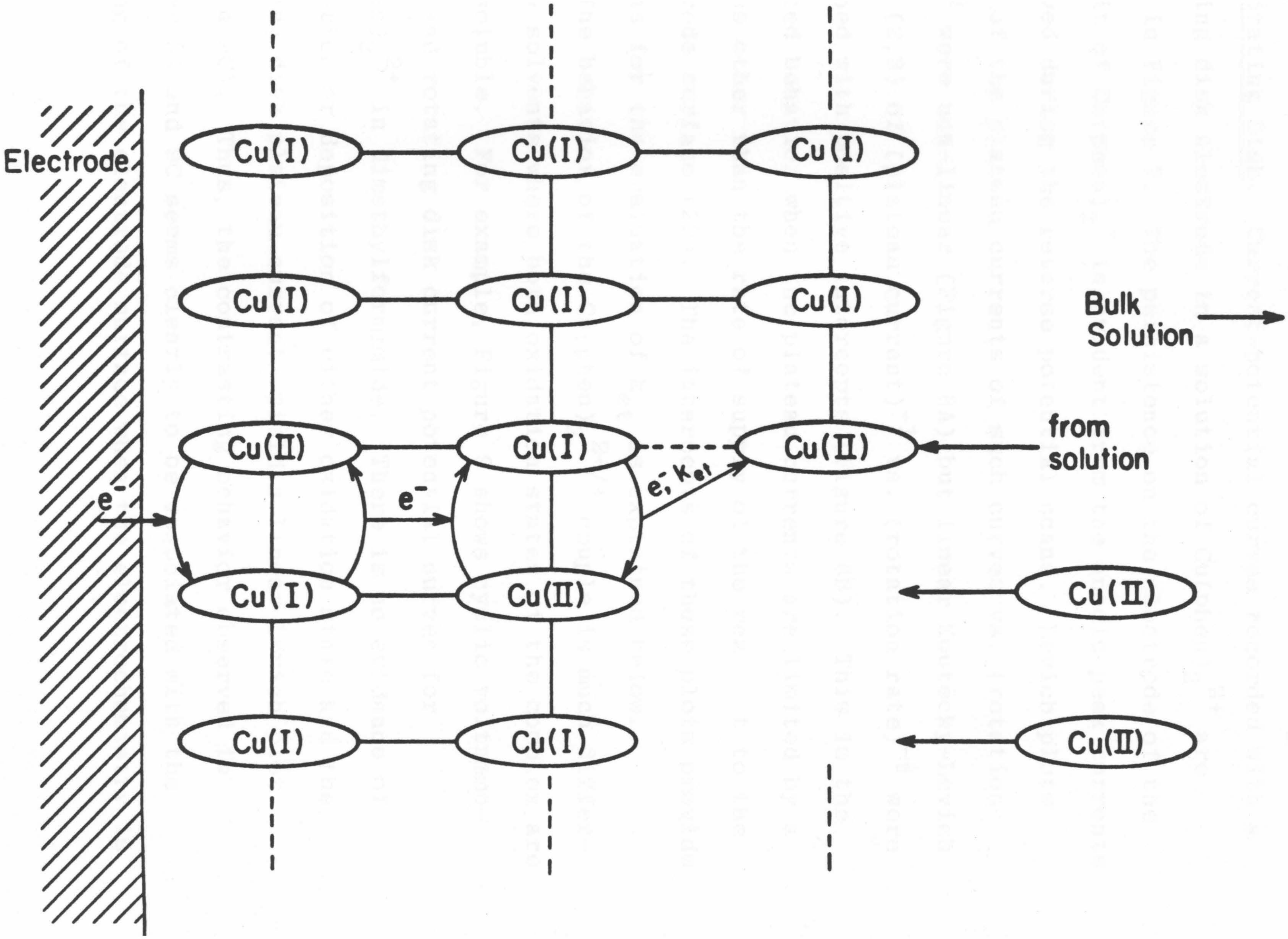


400
300
200
100
Q, μC cm^{-2}
0
0.5
1
Time, sec
D
C
B
A

electrode correspond to multiple layers of the complex. Prolonging the deposition times or increasing the electrode rotation rate or concentration of $Cu(phen)_2^{2+}$ all produce increasingly thick deposits. Nevertheless, the reduction currents at rotating disk electrodes stabilize within a few seconds when the potential is stepped to the limiting current plateau and remain steady for hundreds of seconds as the $Cu(phen)_2^{+}$ coating continues to deposit on the electrode. These features of the rotating disk responses indicate that the thickening film on the electrode introduces no impediment to the flow of current. It therefore seems likely that as the film grows by reductive deposition of additional $Cu(phen)_2^{+}$ at the film/electrolyte interface, the electrons necessary for the reduction are transported from the underlying electrode across the deposit by electron hopping between Cu(I) and Cu(II) centers in the coating.

A schematic model of a possible electron transport process is sketched in Figure 6. Anions must be incorporated in the deposit as it forms to maintain electroneutrality so that the composition of the deposit is assumed to be $Cu(phen)_2Cl$. The rates of electron transfer from the electrode to nearby Cu(II) sites and between Cu(I) and Cu(II) sites in the interior of the deposit are assumed to be rapid. The deposit grows by reduction of $Cu(phen)_2^{2+}$ molecules arriving at the deposit/solution interface at a rate governed by the second-order constant k_{et}. An estimate of the

Figure 6. Schematic model of the mechanism of electron transport through a growing deposit of $Cu(phen)_2^+$ on a graphite electrode. The anions that must also be incorporated in the deposit are not shown.


Electrode
Cu(I)
Cu(I)
Cu(I)
Cu(I)
Cu(I)
Cu(I)
Bulk Solution
Cu(II)
Cu(I)
Cu(II)
from solution
e^-
e^-
$e^-; k_{et}$
Cu(I)
Cu(II)
Cu(II)
Cu(I)
Cu(I)
Cu(II)

magnitude of k_{et} was obtained from measurements with a rotating disk electrode.

Koutecky-Levich Analysis of the Reduction of $Cu(phen)_2^{2+}$ at the Rotating Disk. Current-potential curves recorded with a rotating disk electrode in a solution of $Cu(phen)_2^{2+}$ are shown in Figure 7. The persistence on the electrode of the deposit of $Cu(phen)_2^{+}$ is evident from the anodic peak currents observed during the reverse potential scans. Levich plots (2,3) of the plateau currents of such curves vs. (rotation rate)$^{\frac{1}{2}}$ were non-linear (Figure 8A) but linear Koutecky-Levich plots (2,3) of (plateau current)$^{-1}$ vs. (rotation rate)$^{-\frac{1}{2}}$ were obtained with positive intercepts (Figure 8B). This is the expected behavior when the plateau currents are limited by a process other than the rate of supply of the reactant to the electrode surface (22). The intercepts of these plots provide a means for the evaluation of k_{et} as explained below.

The behavior of the $Cu(phen)_2^{2+/+}$ couple is much different in solvents where both oxidation states of the complex are more soluble. For example, Figure 9 shows cyclic voltammograms and rotating disk current potential curves for $Cu(phen)_2^{2+}$ in dimethylformamide. There is no evidence of adsorption or deposition of either oxidation state and the rotating disk plateau currents yield a linear Levich plot (Figure 9C). Thus, the contrasting behavior observed in Figures 8A and 9C seems clearly to be associated with the coating of the electrode surface when the electrochemistry is

Figure 7. Current potential curves for the reduction of 0.2 mM Cu(phen)$_2^{2+}$ at a rotating graphite disk electrode. Supporting electrolyte: 0.1 M NaCl (pH 5.6). Potential scan rate: 5 mV s^{-1}.

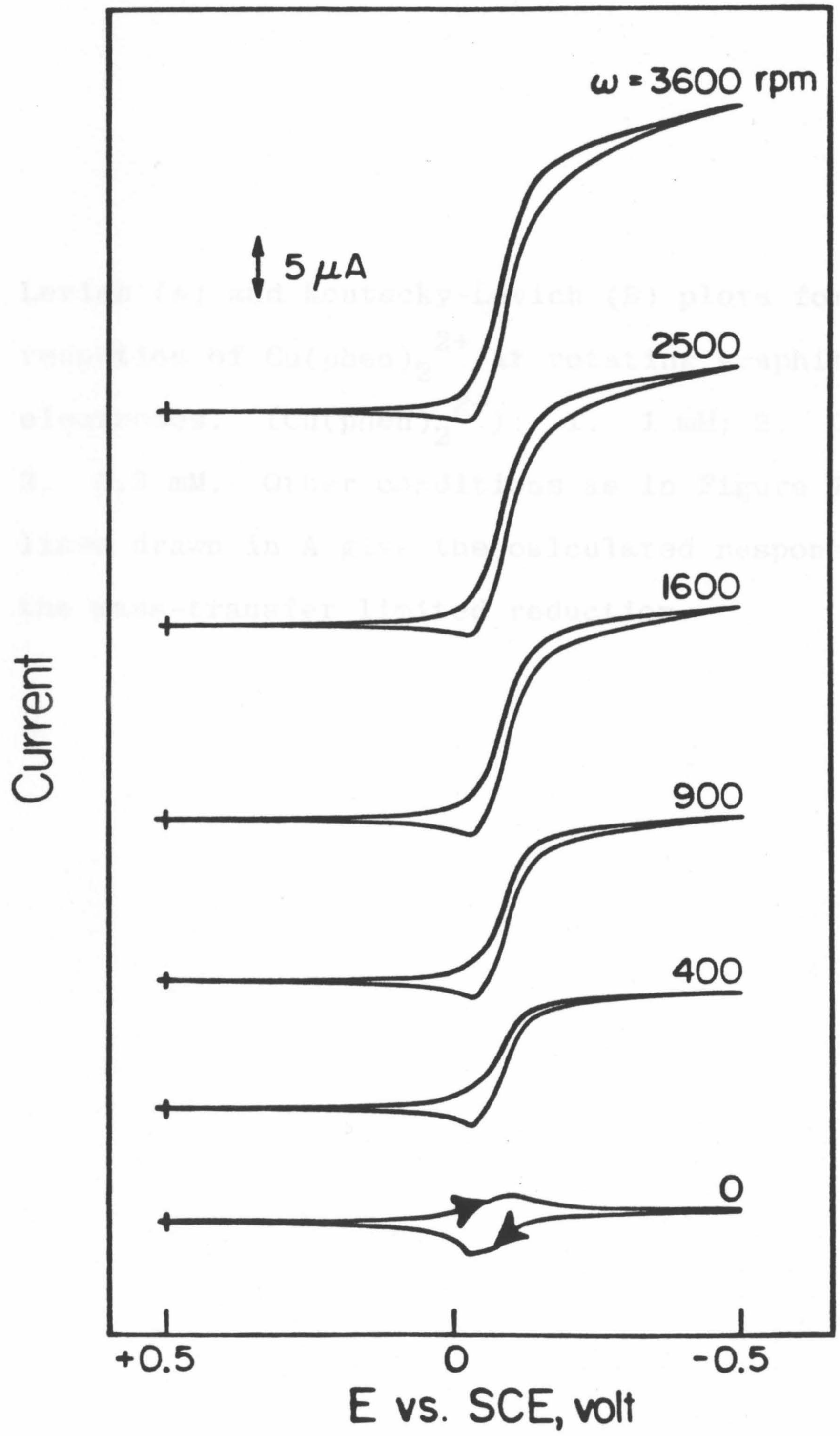

ω = 3600 rpm
5 μA
2500
1600
900
400
0
Current
+0.5
0
-0.5
E vs. SCE, volt

Figure 8. Levich (A) and Koutecky-Levich (B) plots for the reduction of $Cu(phen)_2^{2+}$ at rotating graphite disk electrodes. ($Cu(phen)_2^{2+}$): 1. 1 mM; 2. 0.5 mM; 3. 0.2 mM. Other conditions as in Figure 7. The lines drawn in A give the calculated responses for the mass-transfer limited reduction.

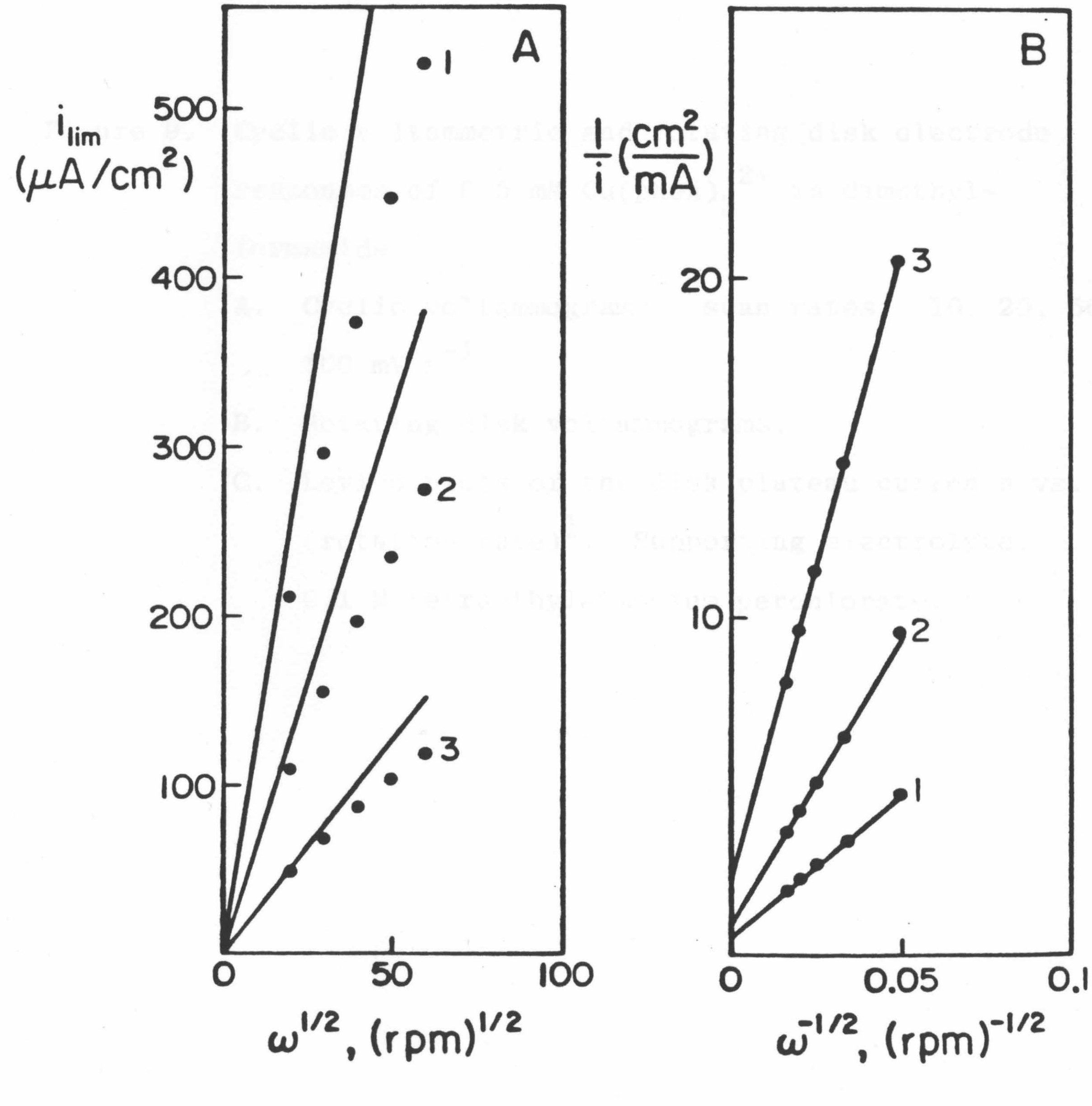
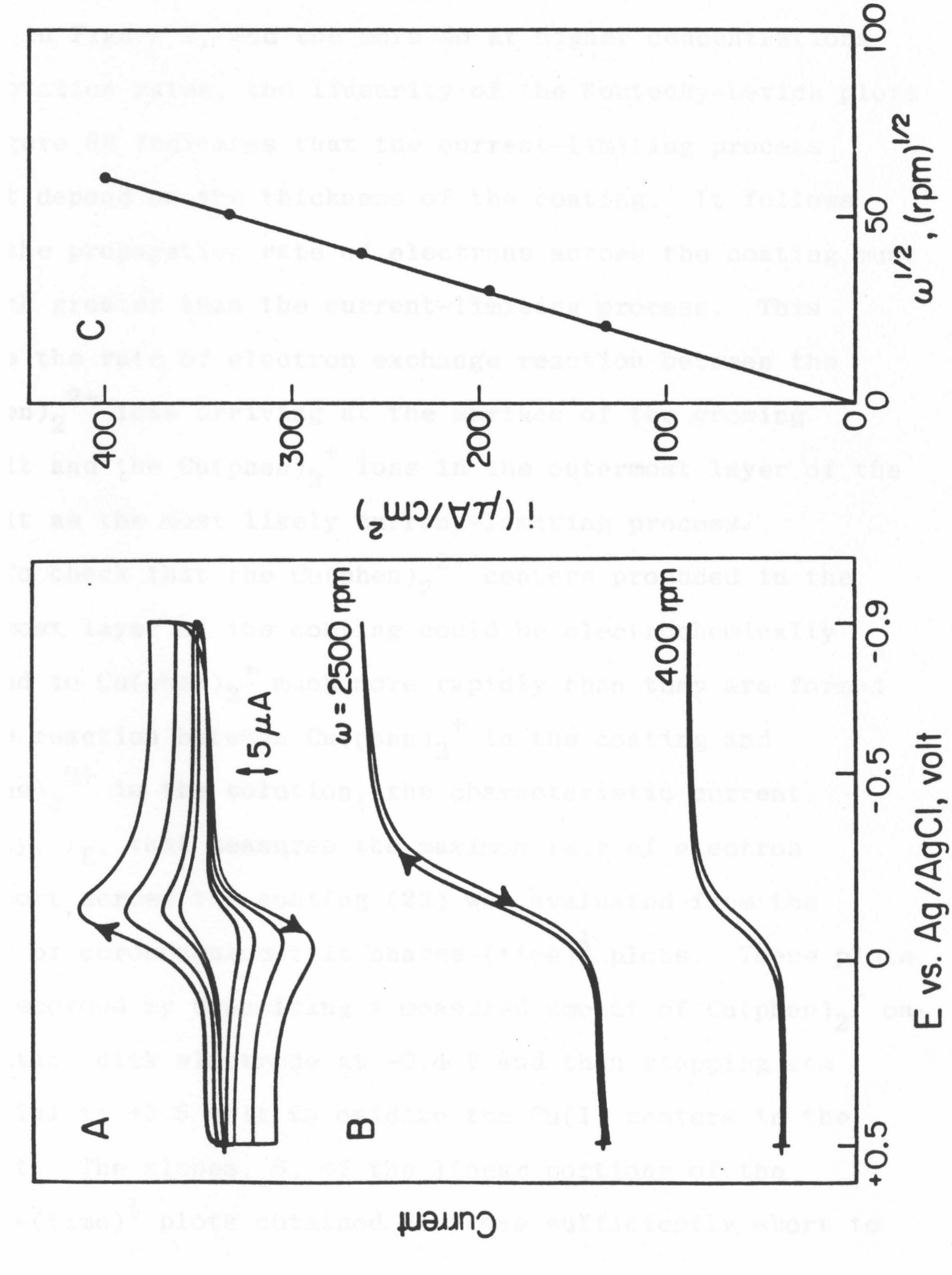

A
i_{lim} (μA/cm^2)
500
400
300
200
100
0
50
100
1
2
3
$\omega^{1/2}$, (rpm)$^{1/2}$
B
$\frac{1}{i}$ ($\frac{cm^2}{mA}$)
20
10
0
0.05
0.1
3
2
1
$\omega^{-1/2}$, (rpm)$^{-1/2}$

Figure 9. Cyclic voltammetric and rotating disk electrode responses of 0.5 mM $Cu(phen)_2^{2+}$ in dimethylformamide.

A. Cyclic voltammograms: scan rates: 10, 20, 50, 100 mV s^{-1}.

B. Rotating disk voltammograms.

C. Levich plots of the disk plateau currents vs. $(\text{rotation rate})^{\frac{1}{2}}$. Supporting electrolyte: 0.1 M tetraethylammonium perchlorate.


A
B
C
5 μA
ω = 2500 rpm
400 rpm
Current
E vs. Ag/AgCl, volt
+0.5
0
-0.5
-0.9
i (μA/cm^2)
0
100
200
300
400
$\omega^{1/2}$, (rpm)$^{1/2}$
0
50
100

examined in aqueous media.

Since the thickness of the deposited layer of $Cu(phen)_2^+$ increases continuously during the recording of curves such as those in Figure 7, and the more so at higher concentrations and rotation rates, the linearity of the Koutecky-Levich plots in Figure 8B indicates that the current-limiting process cannot depend on the thickness of the coating. It follows that the propagation rate of electrons across the coating must be much greater than the current-limiting process. This leaves the rate of electron exchange reaction between the $Cu(phen)_2^{2+}$ ions arriving at the surface of the growing deposit and the $Cu(phen)_2^+$ ions in the outermost layer of the deposit as the most likely current-limiting process.

To check that the $Cu(phen)_2^{2+}$ centers produced in the outermost layer of the coating could be electrochemically reduced to $Cu(phen)_2^+$ much more rapidly than they are formed by the reaction between $Cu(phen)_2^+$ in the coating and $Cu(phen)_2^{2+}$ in the solution, the characteristic current density, i_E, that measures the maximum rate of electron transport across the coating (23) was evaluated from the slopes of chronocoulometric charge-$(time)^{\frac{1}{2}}$ plots. These plots were recorded by depositing a measured amount of $Cu(phen)_2^+$ on a rotating disk electrode at -0.4 V and then stepping its potential to +0.5 volt to oxidize the Cu(I) centers in the deposit. The slopes, S, of the linear portions of the charge-$(time)^{\frac{1}{2}}$ plots obtained at times sufficiently short to obtain a diffusion-like response (24) were used to calculate i_E according to equation (1):

$$i_E = \frac{\pi S^2}{4F\Gamma_{tot}} \qquad (1)$$

where F is the Faraday and Γ_{tot} is the total quantity of $Cu(phen)_2{}^+$ deposited. Since the measured values of S were essentially independent of Γ_{tot}, the value of i_E decreases as Γ_{tot} was increased (Table 1) and the deposit thickened. However, even with 1 mM solutions of $Cu(phen)_2{}^{2+}$ that produced the thickest deposits examined, i_E was always more than an order of magnitude greater than the maximum kinetically limited currents corresponding to the intercepts of the Koutecky-Levich plots. Thus the propagation of electrons through the deposits of $Cu(phen)_2{}^+$ was clearly not a current-limiting process.

Koutecky-Levich plots such as those in Figure 8B were analyzed by means of equations (2) and (3) (24)

$$\frac{1}{i_{lim}} = \frac{1}{i_L} + \frac{1}{i_k} \qquad (2)$$

where i_{lim} is the plateau current of current-potential curves such as those in Figure 7, i_L is the corresponding Levich current (2) which is proportional to $\omega^{\frac{1}{2}}$, and i_k, the kinetic current density, is given by equation (3)

$$i_k = k_{et}FC_A\Gamma_m \qquad (3)$$

where k_{et} is the rate constant governing the reaction between the $Cu(phen)_2{}^+$ in the outermost layer of the coating and

TABLE 1

Chronocoulometric Slopes for the Oxidation of $Cu(phen)_2^+$ Deposited on Graphite Electrodes[a]

Equilibration time,[b] sec.	$10^9 \Gamma_{tot}$,[c] mole cm^{-2}	10^{-3} S[d] $\mu C\ cm^{-2}\ s^{-1}$	i_E[e] mA cm^{-2}
30	2.7	4.0	48
60	-	3.9	-
100	3.7	4.0	35
200	-	4.0	-

a. $Cu(phen)_2^+$ was deposited from a 1 mM solution of $Cu(phen)_2^{2+}$ with the electrode held at -0.4 volt.

b. Length of time that the electrode was held at -0.4 volt to deposit $Cu(phen)_2^+$.

c. Measured by coulometric assay after transfer of the coated electrode to pure supporting electrolyte solution.

d. Slope of plot of charge vs. $(time)^{\frac{1}{2}}$ when electrode potential was stepped to +0.5 volt. The slope was evaluated at times short enough to obtain semi-infinite diffusion-like responses.

e. Calculated from equation (1).

$Cu(phen)_2^{2+}$ in solution (M^{-1} s^{-1}), C_A is the concentration of $Cu(phen)_2^{2+}$ in the bulk of the solution (M) and Γ_m is the quantity of $Cu(phen)_2^+$ in the outermost layer (mole cm^{-2}). The intercepts of lines such as those in Figure 8B were used with equations (2) and (3) to evaluate i_k and the rate constant, k_{et}. Table 2 contains a set of rate constants evaluated for a range of concentrations of $Cu(phen)_2^{2+}$. Γm was taken as 3.3 x 10^{-10} mole cm^{-2} at all concentrations of $Cu(phen)_2^{2+}$. This value was calculated from the dimensions of a molecule of $Cu(phen)_2Cl$ and the electrode roughness factor of 3 estimated from the saturation adsorption measured for $Cu(phen)_2^{2+}$ (vide supra). Substitution of trifluoromethanesulfonate for the chloride supporting electrolyte (fifth entry in Table 2) produced no change in the kinetic response indicating that chloride anions do not play an important role in the electron transfer process. The values of k_{et} obtained are reasonably constant despite the large differences in the total quantities of $Cu(phen)_2^+$ present in the coatings that were used to obtain the data in Table 2. This supports the assumption that only the outermost layer of the $Cu(phen)_2^+$ deposit participates in the reaction with $Cu(phen)_2^{2+}$. The average value of k_{et} is ca. 10^5 M^{-1} s^{-1}.

<u>Oxidation of $Cu(phen)_2^+$ at the Rotating Disk Electrode</u>. The experiments of Figures 7 and 8 were repeated with a solution of $Cu(phen)_2^{2+}$. The experiments were carried out with the apparatus inside a controlled atmosphere box to avoid air

TABLE 2

Evaluation of k_{et} from Rotating Disk Voltammetric Data[a]

$Cu(phen)_2^{2+}$, M	Int.,[b] cm^2/mA	$10^5\ k_{et}\ \Gamma_m$,[c] $cm^{-2}\ s^{-1}$	$10^{-4}\ k_{et}$[d] $M^{-1}\ s^{-1}$
1.0×10^{-3}	0.53	2.0	6.1
5.0×10^{-4}	0.96	2.2	6.7
2.0×10^{-4}	2.3	2.3	7.0
1.0×10^{-4}	4.3	2.4	7.3
1.0×10^{-4}[e]	4.2[e]	2.5[e]	7.5[e]
5.0×10^{-5}	11	1.9	5.8
2.0×10^{-5}	15	3.5	11
		Avg:	7.3

a. Supporting electrolyte: 0.1 M NaCl (pH 5.6).

b. Intercept of Koutecky-Levich plot (Figure 8B); Int. = i_k^{-1}.

c. From equation (3).

d. Calculated by taking $\Gamma_m = 3.3 \times 10^{-10}$ mole cm^{-2} in every case.

e. Supporting electrolyte: 0.1 M CF_3SO_3Na (pH 5.6).

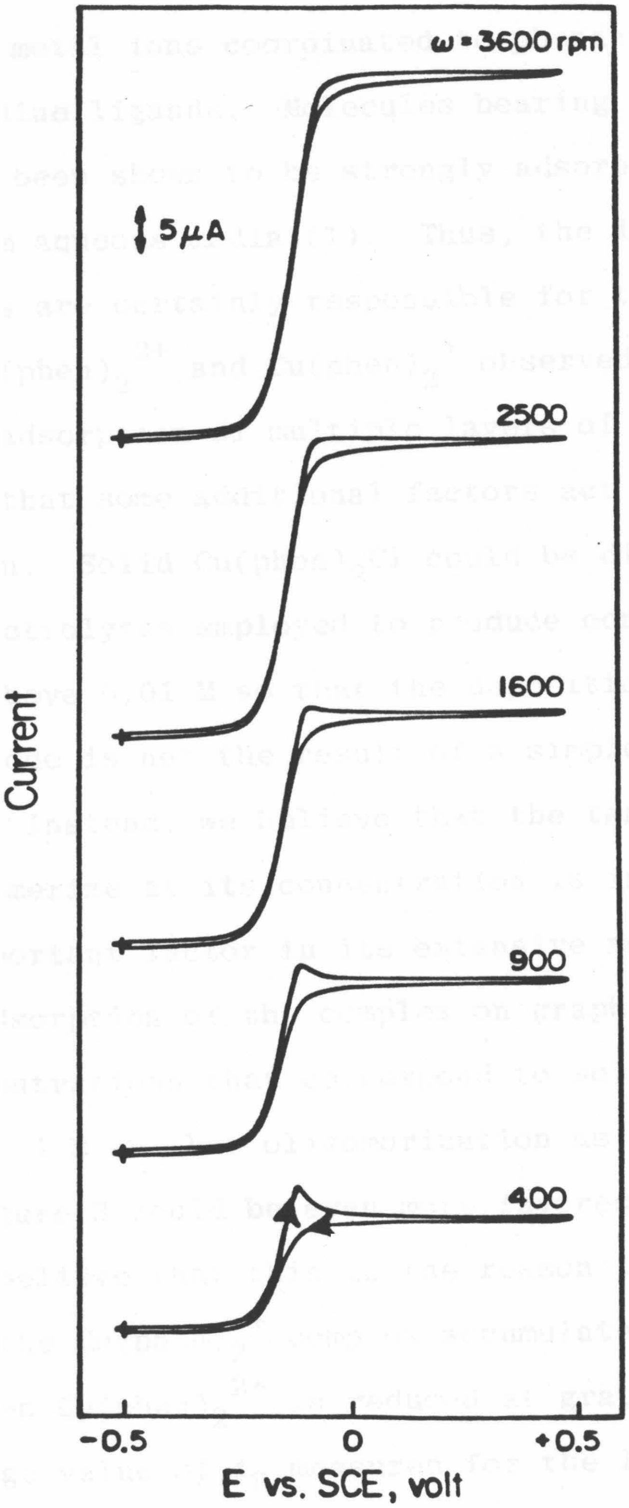
oxidation of the $Cu(phen)_2^+$. The current potential curves obtained are shown in Figure 10. The peak preceding the current plateau at the lower rotation rates results from the oxidation of the initially adsorbed complex. In contrast with the analogous curves in Figure 7, a Levich plot of the plateau currents for the curves in Figure 10 remains linear up to the highest accessible rotation rate.

We expected to obtain results in these experiments that resembled those in Figures 7 and 8 because the same self-exchange reaction between $Cu(phen)_2^{2+}$ and $Cu(phen)_2^+$ could limit the current. Instead, the currents were limited only by the supply of $Cu(phen)_2^+$ to the electrode. The difference in behavior seems most likely to be the result of the much thinner coating of adsorbed complex that is present on the electrode surface when $Cu(phen)_2^+$ is oxidized to $Cu(phen)_2^{2+}$. Adsorption of the latter complex amounts to no more than a single monolayer through which electrons can pass directly without the necessary intervention of the self-exchange reactions. The kinetics of the latter only begin to limit the flow of current when the thicker coatings resulting from the deposition of $Cu(phen)_2Cl$ prevent direct electron transfer between the graphite electrode and the reactant.

DISCUSSION

The adsorption of the 1,10-phenanthroline complexes of Cu(I) and Cu(II) on graphite surfaces is not surprising.

Figure 10. Current potential curves for the oxidation of 0.4 mM $Cu(phen)_2^+$ at a rotating graphite disk electrode. Experimental conditions as in Figure 7.


ω = 3600 rpm
5 μA
2500
1600
900
400
Current
−0.5
0
+0.5
E vs. SCE, volt

Qualitative polarographic evidence of such adsorption on mercury electrodes was reported many years ago (25) and in numerous subsequent studies, adsorption has complicated the cyclic voltammetry of metal ions coordinated to phenanthroline, terpyridine or bipyridine ligands. Molecules bearing aromatic centers have recently been shown to be strongly adsorbed on graphite surfaces from aqueous media (1). Thus, the 1,10-phenanthroline ligands are certainly responsible for the adsorption of both $Cu(phen)_2^{2+}$ and $Cu(phen)_2^{+}$ observed in this study. However, the adsorption of multiple layers of $Cu(phen)_2^{+}$ indicates that some additional factors act to enhance its adsorption. Solid $Cu(phen)_2Cl$ could be dissolved in the supporting electrolytes employed to produce concentrations of Cu(I) well above 0.01 M so that the deposition of complex on the electrode is not the result of a simple precipitation reaction. Instead, we believe that the tendency of this complex to oligomerize at its concentration is increased (Figure 3) is the important factor in its extensive adsorption. The ligand-induced adsorption of the complex on graphite yields cross-sectional concentrations that correspond to solution concentrations of ca. 1 M so that oligomerization as suggested by the spectra in Figure 3 would be even more favored in the adsorbed state. We believe that this is the reason that large quantities of the $Cu(phen)_2^{+}$ complex accumulate on the electrode surface when $Cu(phen)_2^{2+}$ is reduced at graphite electrodes. The large value of i_E measured for the layer of oligomerized $Cu(phen)_2^+$ indicates good "electronic conductivity" in the vicinity of the $Cu(phen)_2^{2+/+}$ redox potential so that the layer of oligomerized complex is able to grow continuously as $Cu(phen)_2^{2+}$ is reduced to $Cu(phen)_2^+$ at the outer edge of the layer.

Rate Constant for Self-Exchange. The average value of k_{et} listed in Table 2 does not agree with either of the mutually discrepant previous estimates of the rate constant for self-exchange between $Cu(phen)_2^{2+}$ and $Cu(phen)_2^+$ in homogeneous solution: $k_{ex} = 5 \times 10^7\ M^{-1}\ s^{-1}$ (5a) and $k_{ex} = 50\ M^{-1}\ s^{-1}$ (5b). It is not difficult to find reasons for expecting the rate of electron self-exchange between two reactants to be governed by a different rate constant when the reaction proceeds in homogeneous solution than when one of the co-reactants is bound to a surface in an oligomerized state. Steric factors that decrease the rate of collision between the co-reactants when one is confined to the surface as well as differences in the activation energies for the two reaction conditions could act to decrease the rate constant for the surface reaction.

The previously reported values for the homogeneous self-exchange reaction (5) were both obtained from the measured rates of cross-reactions (rather than the self-exchange reaction itself) combined with the Marcus correlation of rate constants for the two types of reactions (26). However, there is good reason for doubting the applicability of the unmodified

Marcus correlation between cross- and self-exchange reactions that involve structural changes as significant as those that both halves of the $Cu(phen)_2^{2+/+}$ couple undergo upon electron transfer (27). Indeed, the previous discrepant estimates of k_{ex} for the $Cu(phen)_2^{2+/+}$ couple have been shown to be mutually compatible with a value of k_{ex} near 10^5 M^{-1} s^{-1} if the Marcus correlation is appropriately modified to take account of the possible effects of large structural changes (27). The resulting agreement between the values of k_{ex} obtained from (re-calculated) homogeneous and heterogeneous experiments suggests that despite the adsorption and oligomerization of $Cu(phen)_2^{+}$ on the surface of graphite electrodes its reactivity in undergoing electron exchange with $Cu(phen)_2^{2+}$ in solution is not significantly changed.

CONCLUSIONS

The adsorption behavior of the bis-1,10-phenanthroline complexes of Cu(II) and Cu(I) at pyrolitic graphite electrodes was studied. The adsorption of the Cu(II) complex reaches a full monolayer at a concentration of ca. 0.1 mM. The Cu(I) complex appears to oligomerize both in solution and in the adsorbed layer. As a result, much larger quantities of the Cu(I) complex can be deposited on the electrode surface. Taking advantage of this fact, rotating disk voltammetric measurements of the reduction of $Cu(phen)_2^{++}$ at electrodes coated with a deposit of the Cu(I) complex were utilized to measure the rate of electron transfer between the two complexes. An estimate of ca. 10^5 M^{-1} S^{-1} was obtained for the rate constant governing the self-exchange reaction.

Although the portion of the catalytic current of dioxygen reduction by $Cu(phen)_2^+$ adsorbed on graphite electrode surfaces was not separable due to low signal-to-noise ratio, some comments on the catalysis of electroreduction of molecular oxygen may be feasible based on the information given here. The two contrasting facts of little changed chemical reactivity in the electron transfer reaction of the adsorbed Cu(I) complex compared to the solution Cu(I) and its apparent low catalytic activity toward dioxygen reduction may suggest that hydrogen peroxide produced in the reaction destroys the catalyst. In some cases (28), 1,10-phenanthrolines are known to react with hydrogen peroxide to produce N-oxides. Also, the previous work by Shigehara and Anson showed that the catalytic activity of $Cu(phen)_2^+$ rapidly decays at electrode surfaces (4). The catalytic studies, however, will be still possible at the surfaces of electrode materials with small backgrounds. In any case, simple copper complexes may not be desirable electrocatalysts for the reduction of molecular oxygen because the reduction produces hydrogen peroxide as a major product, similar to other single metal catalyst systems (29). In this regard, copper clusters or catalysts with multicopper atoms may be worth studying as electrocatalysts for the reduction of dioxygen. The next chapter describes an electrocatalytic system based on multicopper atoms.

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CHAPTER III

CATALYSIS OF THE REDUCTION OF DIOXYGEN AT GRAPHITE ELECTRODES COATED WITH FUNGAL LACCASE A

INTRODUCTION

As part of continuing studies of potential electrocatalysts for the reduction of dioxygen (1-4) we have tested the catalytic activity of fungal laccase A adsorbed on the surface of freshly polished graphite electrodes. This copper-containing enzyme is known to operate efficiently in homogeneous solution as a catalyst for the oxidation of inorganic and organic substrates by dioxygen (5). Oxidizable substrates include those whose formal potentials are almost as positive as that of the redox active copper sites in the enzyme (5). In all cases the catalyzed reactions accomplish the four-electron reduction of dioxygen to water. If the enzyme were able to operate with comparable facility when attached to electrode surfaces it could be an attractive electrocatalyst for dioxygen reduction. Previous studies of laccase A as a potential electrocatalyst for dioxygen reduction by Tarasevich and co-workers (6) have indicated some success but extensive data obtained under fully specified experimental conditions are lacking. In the present work the direct electroreduction of dioxygen catalyzed by laccase A at graphite electrodes is examined in the absence of redox mediators and the reduction is shown to proceed quantitatively to water. Conditions were found where a reversible electrochemical response from laccase A could be observed in the absence of dioxygen. The electroactivity of the enzyme appears at a potential that is very close to the potential where the catalyzed reduction of dioxygen occurs suggesting that the former is responsible for the latter. The results obtained allow several observations to be offered concerning the utility of the enzyme as an electrocatalyst.

EXPERIMENTAL

Materials. The fungal laccase A employed was from *Polyporous versicolor* and its isolation and purification have been previously described (7). The purified enzyme was stored as a frozen solution at -60°C. Enzyme concentrations were determined from the absorbance at 610 nm using $\varepsilon = 4900\ M^{-1}\ cm^{-1}$ as the molar extinction coefficient (8). 4,4'-bipyridine and 2,9-dimethylphenanthroline (Aldrich) were recrystallized three times from water before use. Other reagent grade chemicals were used as received. Solutions were prepared with distilled water that was further treated by passage through a purification train (Barnstead Nanopure). Supporting electrolytes consisted of 0.1 M $NaClO_4$ plus appropriate buffers (pH 2.4-3.1, 0.04 M phosphate; pH 3.3, 0.04 M acetate, pH 3.5-5.5, 0.02 M acetate). Electrodes were prepared from pyrolytic graphite (Union Carbide Co.) mounted so that the edges of the graphite planes were exposed to the electrolyte. The electrodes were sealed with heat-shrinkable polyolefin into the end of a stainless stell tube that was attached to the shaft of a commercial electrode rotator (Pine Instrument Co.). The 0.34 cm^2 electrode surface was polished with No. 600 silicon carbined paper.

Apparatus and Procedures. Electrochemical experiments employed a conventional two-compartment cell and appropriate combinations of PAR (EG & G Instrument Co.) or Pine Instrument Co. electronic instruments. Spectra were obtained with a Hewlett-Packard 8450A spectrophotometer. Solutions were saturated with dioxygen or deaerated with pre-purified argon. Experiments were conducted at the ambient laboratory temperature (22 ± 2°C). Potentials were measured and are reported with respect to a sodium chloride saturated calomel electrode (SSCE).

Enzyme coatings were applied to electrodes by delivering to the freshly polished electrode surface two microliter aliquots of stock solutions of the enzyme having concentrations adjusted to provide the quantity of the enzyme it was desired to deposit on the surface. (This procedure yielded more reproducible behavior than resulted when different aliquot volumes of a single stock solution were employed.) The liquid layer was spread across the entire surface by careful motion of the micropipet tip and the electrode was transferred without washing directly to the test solution. Subsequent experiments were initiated in less than 60 seconds. Electrodes were re-polished and re-coated before each experimental run.

For comparison of measured dioxygen reduction currents at rotating graphite disk electrodes with that calculated from the Levich equation (9) the following parameters were employed. Solubility of dioxygen in 0.1 M $NaClO_4$ (pH 3.8): 1.2 mM; diffusion coefficient of O_2: 1.7×10^{-5} cm^2 s^{-1}; kinematic viscosity of water: 0.01 cm^2 s^{-1}.

RESULTS

Electrochemical Response of Laccase in the Absence of Dioxygen. Graphite electrodes coated with laccase as described in the Experimental section exhibit cyclic voltammograms that are indistinguishable from the background voltammograms obtained in the absence of enzyme (Figure 1, curves A and B). However, the presence of laccase on the electrode surface is readily demonstrated by the reduction of dioxygen at potentials as positive as 0.5 volt as coated electrodes (vide infra). An electrochemical response from laccase coatings can be obtained in the absence of dioxygen if 2,9-dimethylphenanthroline (DMP) is added to the supporting electrolyte. Curve C in Figure 1 is a voltammogram for a laccase coated electrode recorded in a solution saturated with DMP (ca. 0.1 mM). The reversible couple that appears near 0.4 volt is not associated with the DMP which is electroinactive in this range of potentials

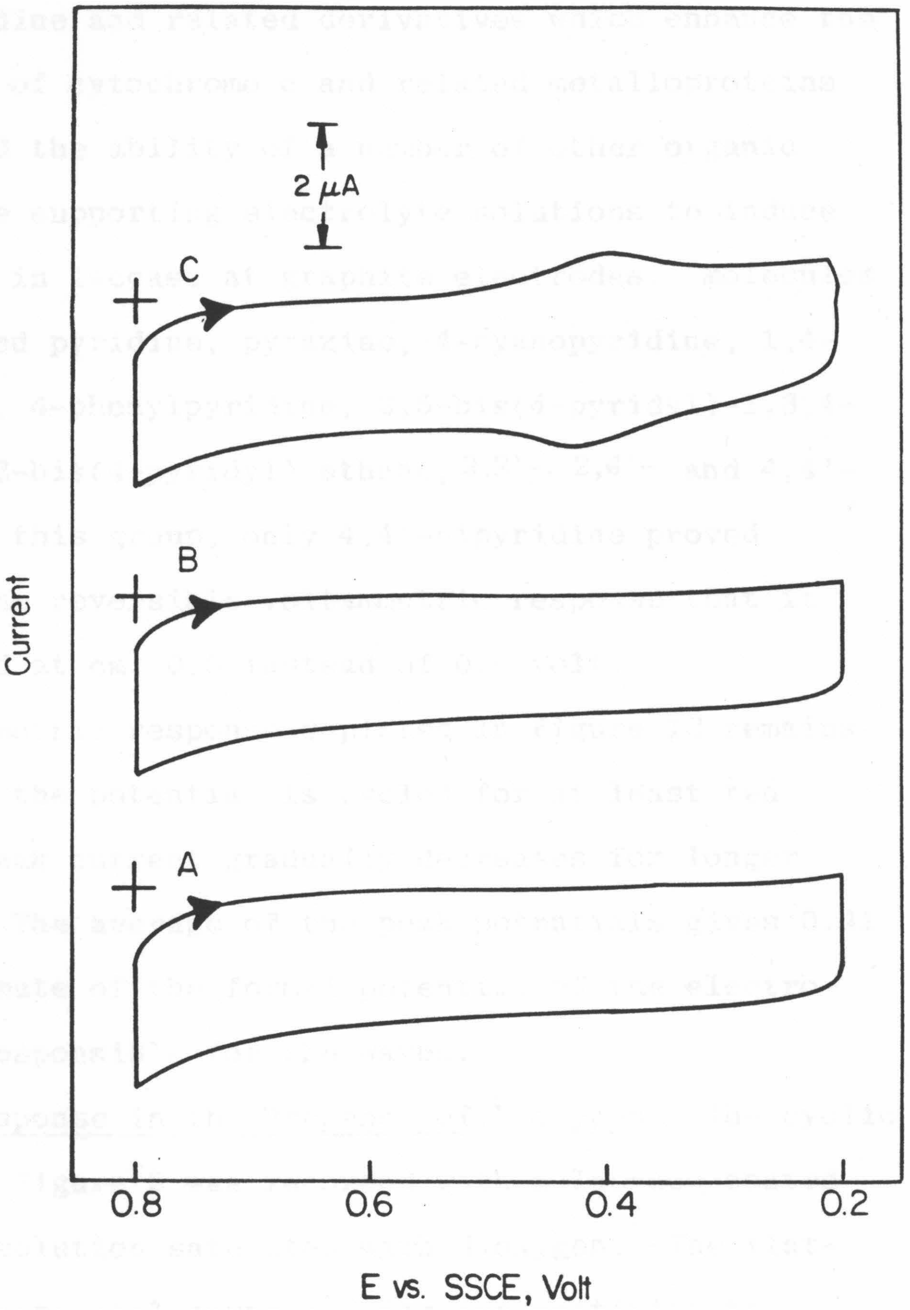
Figure 1. Steady state cyclic voltammograms for edge plane pyrolytic graphite electrodes in the absence of dioxygen. Supporting electrolyte: 0.1 M $NaClO_4$ at pH 5.5.

A. Uncoated electrode in a solution saturated with 2,9-dimethylphanthroline (DMP).

B. Electrode coated with 5×10^{-9} mole cm^{-2} of laccase; supporting electrolyte solution contained no DMP.

C. B except solution was saturated with DMP.

Scan rate: 10 mV s^{-1}.

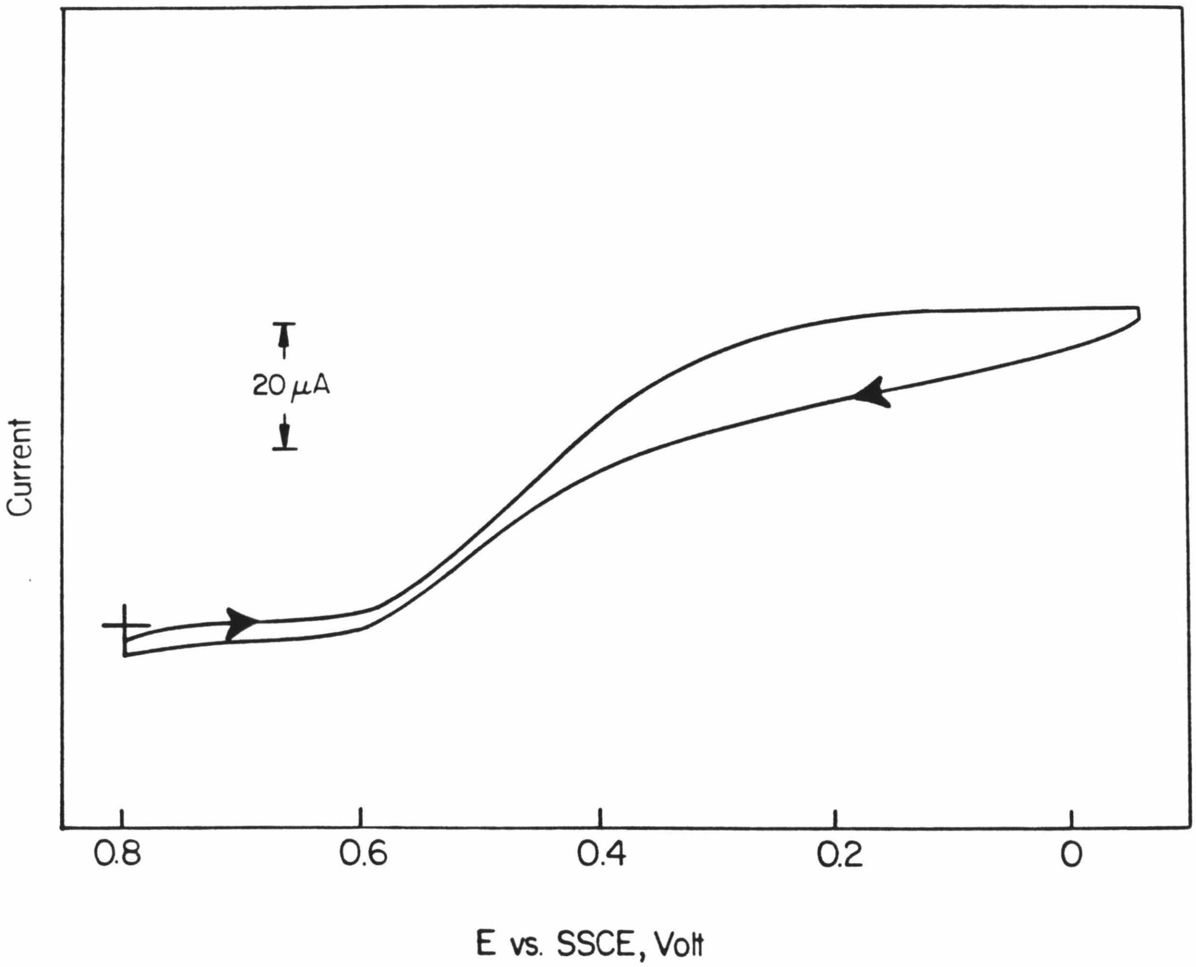

2 μA
C
B
A
Current
0.8
0.6
0.4
0.2
E vs. SSCE, Volt

(Curve A). The mechanism by which the added DMP bestows electroactivity upon the laccase is unclear but its effectiveness is similar to that reported by Hill and co-workers (10) for 4,4'-bipyridine and related derivatives which enhance the electroactivity of cytochrome c and related metalloproteins (11). We tested the ability of a number of other organic molecules in the supporting electrolyte solutions to induce electroactivity in laccase at graphite electrodes. Molecules examined included pyridine, pyrazine, 4-cyanopyridine, 1,4-dicyanopyridine, 4-phenylpyridine, 2,5-bis(4-pyridyl)-1,3,4-thiadiazole, 1,2-bis(4-pyridyl) ethane, 2,2'-, 2,4'- and 4,4'-bipyridine. Of this group, only 4,4'-bipyridine proved effective but the reversible voltammetric response that it induced appeared at ca. 0.5 instead of 0.4 volt.

The voltammetric response depicted in Figure 1C remains undiminished as the potential is cycled for at least ten minutes. The peak current gradually decreases for longer cycling times. The average of the peak potentials gives 0.41 volt as an estimate of the formal potential of the electroactive couple responsible for the waves.

<u>Voltammetric Response in the Presence of Dioxygen</u>. The cyclic voltammogram in Figure 2 was recorded with a laccase-coated electrode in a solution saturated with dioxygen. The flat-topped current-potential curve and its insensitivity to changes in the potential scan rate indicate that a chemical reaction limits the maximum rate at which the dioxygen is

Figure 2. Cyclic voltammogram for the reduction of dioxygen at a laccase-coated electrode. Supporting electrolyte: 0.1 M $NaClO_4$ at pH 3.1 saturated with O_2. Scan rate: 10 mV s^{-1}.


20 μA
Current
0.8
0.6
0.4
0.2
0
E vs. SSCE, Volt

reduced. When the rate of the chemical reaction is low enough compared with the rate at which dioxygen can be diffusely supplied to the electrode there is little or no depletion of dioxygen at the electrode surface so that a flat, instead of a peaked current-potential response is obtained (12).

The response observed for the catalytic reduction of dioxygen is less stable than that for the enzyme itself in the absence of dioxygen (Figure 1C). A second voltammetric scan (at 10 mV s^{-1}) under dioxygen yields a current only two-thirds as large as that obtained during the initial scan. Addition of DMP to the solution diminishes the catalytic response but has no effect on the rate of its decay. To characterize the catalytic responses under standard conditions all experiments were conducted in the absence of DMP with electrodes that had been polished and freshly coated with enzyme.

The potential at which the laccase coating begins to catalyze the reduction of dioxygen in Figure 2, ca. 0.5 volt, is not far from the value where the enzyme exhibits its own electrochemical response in dioxygen-free solutions of DMP, ca. 0.4 volt (Figure 1C). This suggests that the redox process responsible for the waves in Figure 1C is also involved in the catalytic cycle that leads to the reduction of dioxygen.

The magnitudes of the catalytic reduction currents were measured as a function of the quantity of laccase in the aliquots transferred to the electrode. The results (Figure 3) show that the catalytic current saturates when ca. 6×10^{-11} mole cm^{-1} of enzyme are contained in the solution that is transferred to the electrode surface during the coating step. This would amount to multiple layers of a molecule as large as laccase A (M.W. = 65000 (7)). However, the saturation value in Figure 3 is probably an overestimate because it ignores surface roughness and it is unlikely that all of the enzyme in the 2 microliter aliquots to which the electrode is exposed is retained by the surface when the electrode is transferred to the supporting electrolyte solution.

Dioxygen Reduction at Laccase-Coated Rotating Disk Electrodes. When rotating graphite disk electrodes were coated with laccase as described in the Experimental section and used for the reduction of dioxygen the initial plateau currents at the disk decreased continuously for the first 30 minutes. However, after this period when the current had fallen to about one-half of its initial value it became much steadier, allowing the rotation rate dependence of the steady currents to be inspected. In order to conduct the experiments as rapidly as possible the full current-potential curves were not recorded. Instead, the disk electrode potential was set at 0 volt and the rotation rate dependence of the resulting currents was measured. Figure 4 shows a set of data in which the electrode

Figure 3. Variation in the plateau current of cyclic voltammograms for the reduction of dioxygen at a graphite electrode coated with increasing quantities of laccase. Supporting electrolyte: 0.1 M $NaClO_4$ at pH 3.8 saturated with O_2. Scan rate: 10 mV s^{-1}.

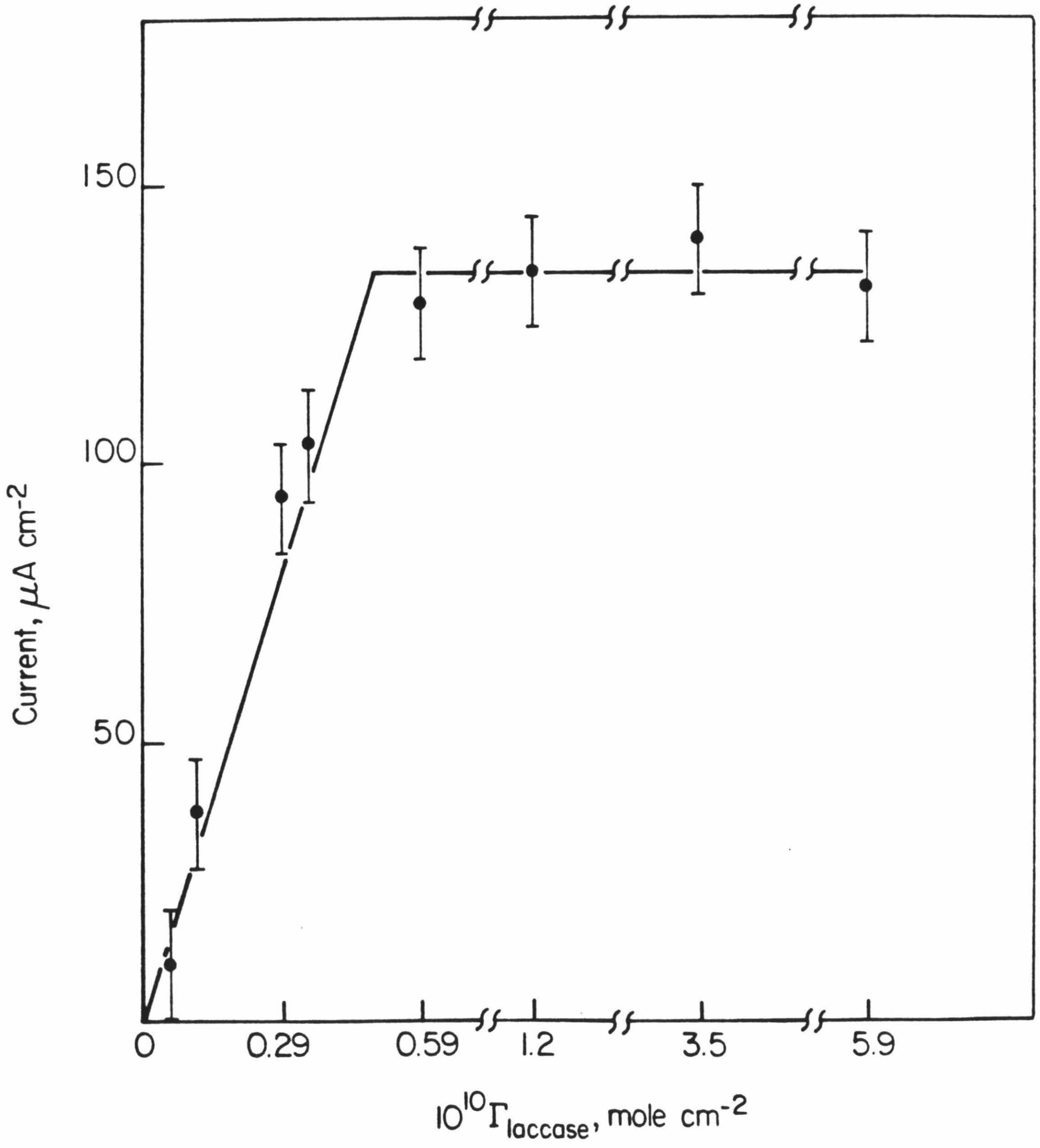
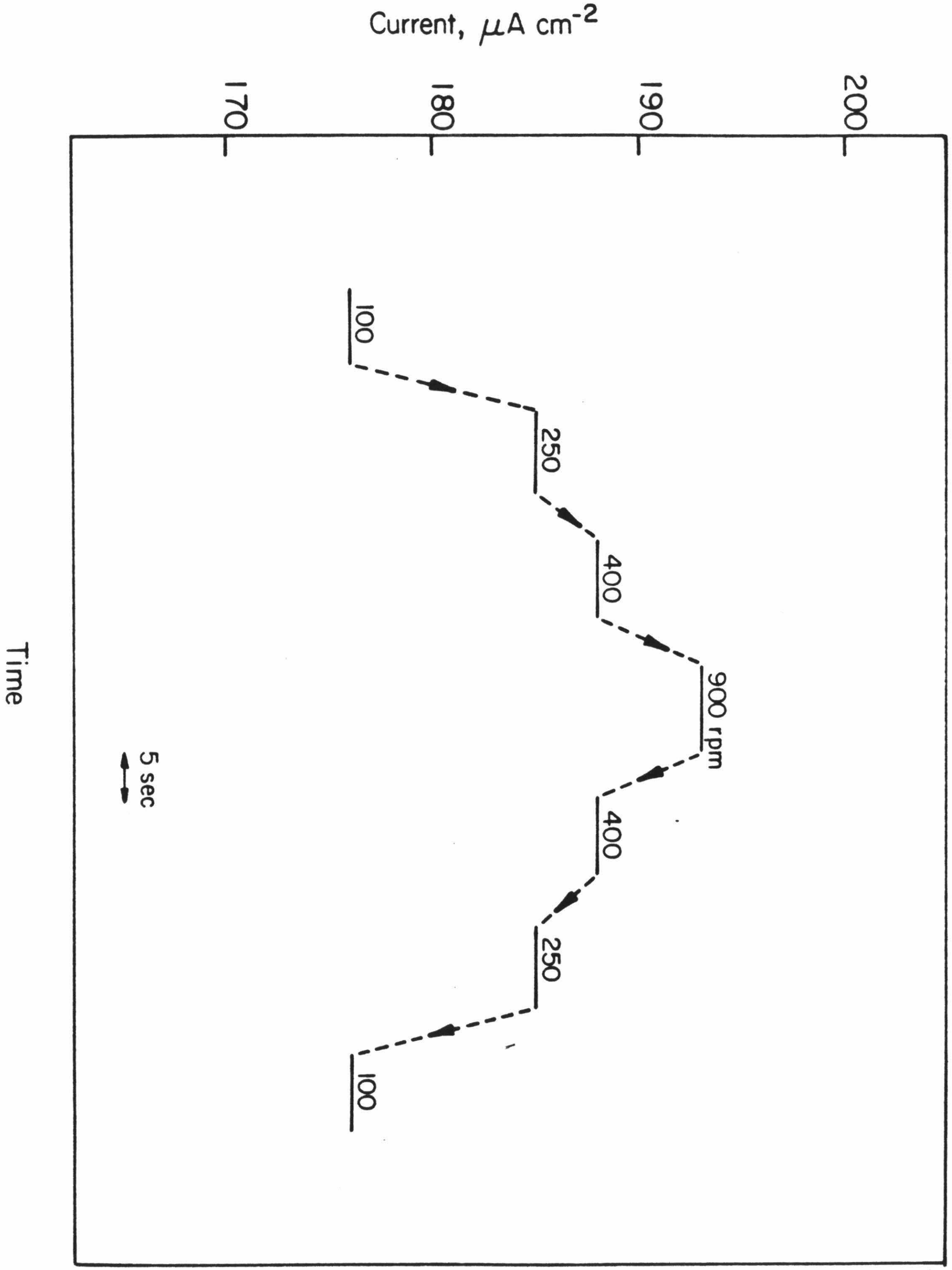

150
100
50
Current, μA cm^{-2}
0
0.29
0.59
1.2
3.5
5.9
$10^{10}\Gamma_{laccase}$, mole cm^{-2}

Figure 4. Plateau currents for the reduction of dioxygen at a rotating graphite disk electrode coated with laccase and maintained at 0 volt vs SSCE. The electrode rotation rates, listed in the Figure, were increased and then decreased in the order shown. Supporting electrolyte: as in Figure 3.


Current, μA cm^{-2}
200
190
180
170
100
250
400
900 rpm
400
250
100
5 sec
Time

rotation rate was increased and then decreased in successive steps. The stability of the laccase coating is indicated by the agreement between the initial and final currents at 100 rpm. The steady limiting disk currents of Figure 4 are presented in the form of a Levich plot (9) in Figure 5A along with the straight line that would have been obtained if the dioxygen were reduced by four electrons at the diffusion controlled rate. The small currents, almost independent of rotation rate, were to be expected on the basis of the flat-topped cyclic voltammetric curve shown in Figure 2. In both experiments the reduction proceeds at a rate that results in rather little depletion of the dioxygen at the electrode surface.

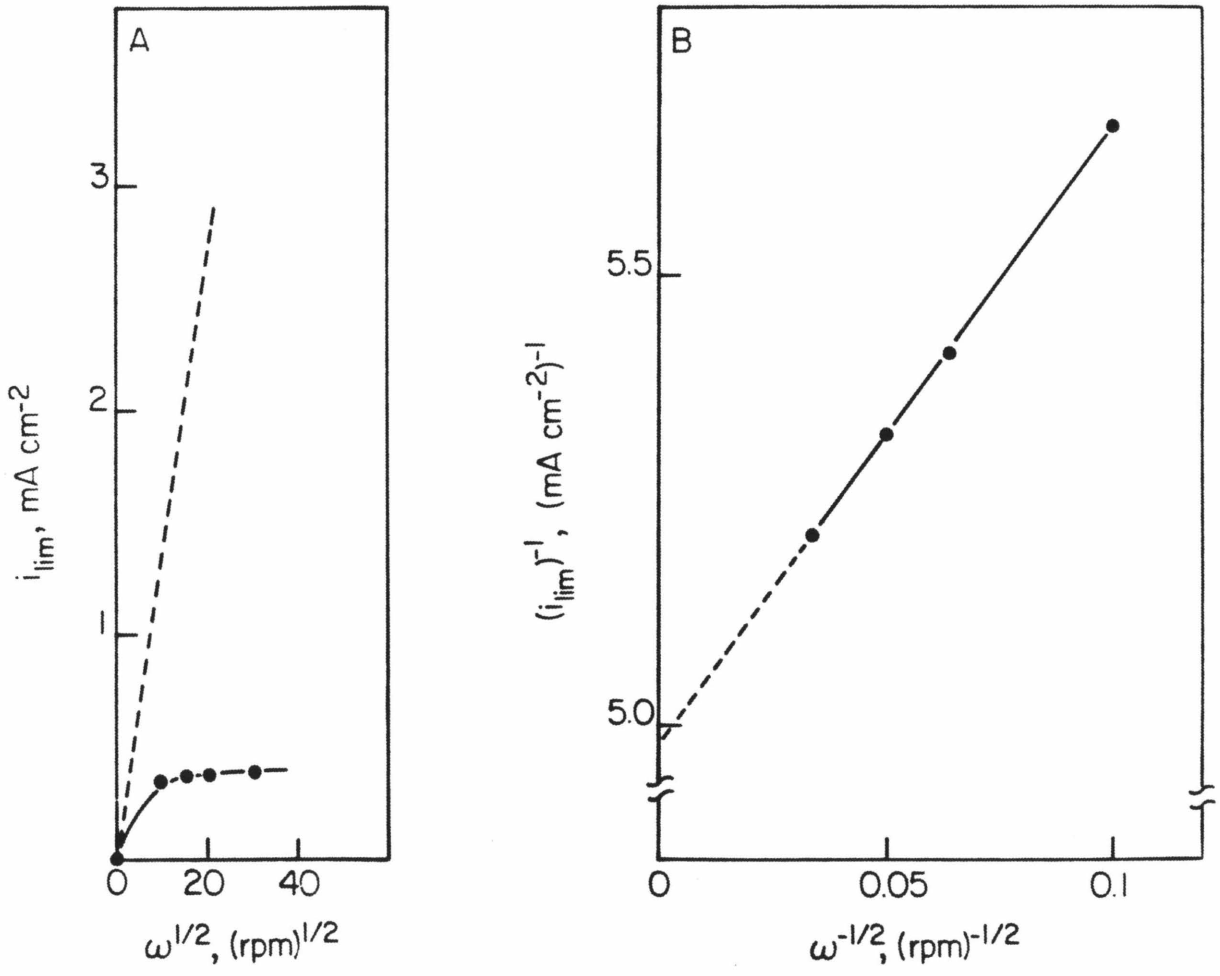
In Figure 5B the same data are shown in the form of a Koutecky-Levich plot (13) of the reciprocal disk plateau currents vs. (rotation rate)$^{-\frac{1}{2}}$. The intercepts of such plots are related to the rate constant of the catalyzed reaction according to equation (1) (13):

$$\frac{1}{i_{lim}} = \frac{1}{i_{lev}} + \frac{1}{i_k} \qquad (1)$$

i_{lim} is the measured plateau current density at the disk, i_{lev} is the calculated diffusion limited Levich current density for the reduction of dioxygen at the rotating disk which is given by the Levich equation (9), and i_k is the kinetic current density given by

$$i_k = kn\,F\,\Gamma C^b \qquad (2)$$

Figure 5. Levich (A) and Koutecky-Levich (B) plots of the data of Figure 4.

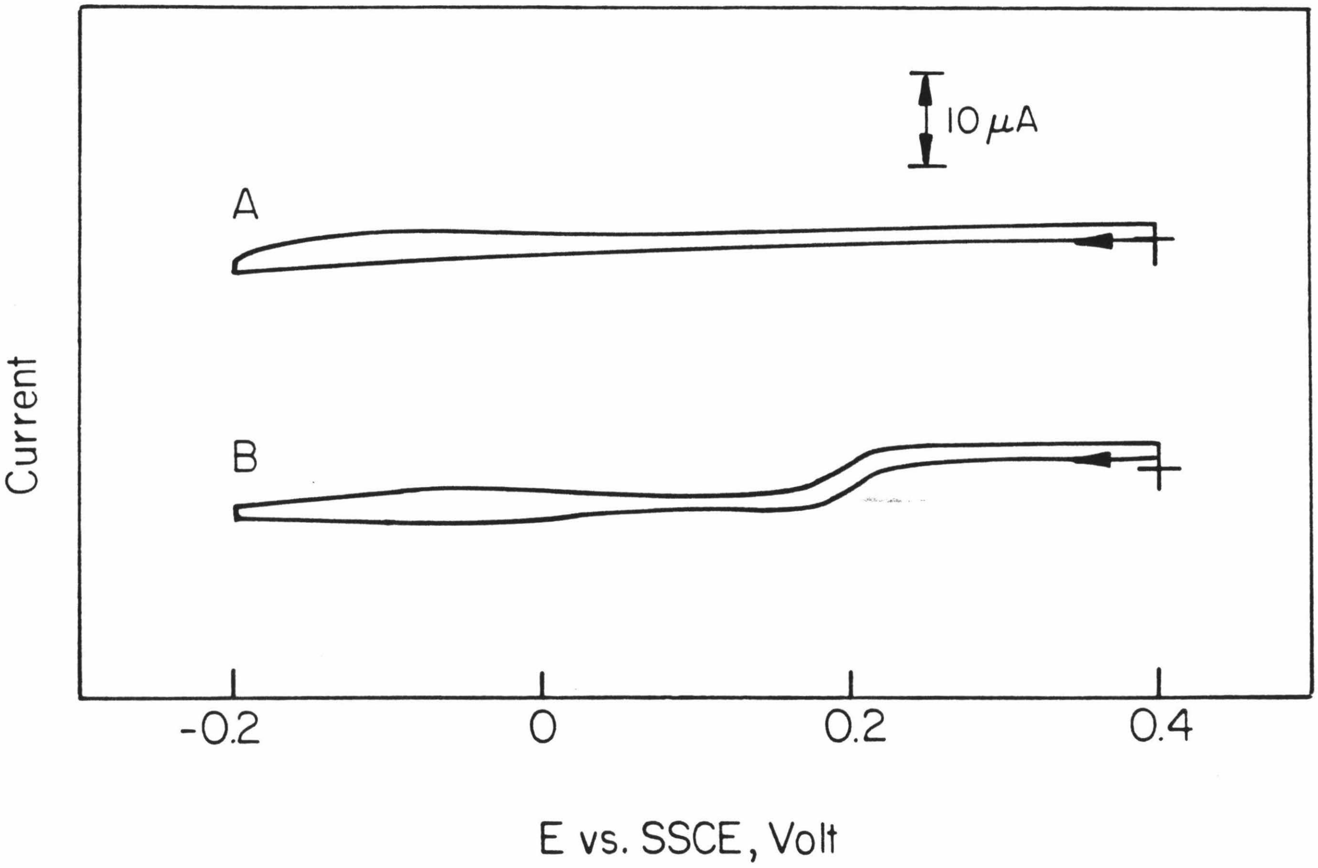

A
3
2
1
0
i_{lim}, mA cm^{-2}
0
20
40
$\omega^{1/2}$, (rpm)$^{1/2}$
B
5.5
5.0
$(i_{lim})^{-1}$, (mA cm^{-2})$^{-1}$
0
0.05
0.1
$\omega^{-1/2}$, (rpm)$^{-1/2}$

where k is the second-order rate constant for the reaction between the enzyme and dioxygen that leads to its catalyzed reduction, n is the number of electrons involved in the reduction, F is the Faraday, Γ is the quantity of catalyst immobilized in the electrode surface (mole cm^{-2}) and C^b is the concentration of dioxygen in the solution.

The Levich current, i_{lev}, is proportional to the square-root of the electrode rotation rate (9) so that the slope of the Koutecky-Levich plots can be used to calculate the Levich current. The slope of the line in Figure 5B matches that calculated for the four-electron reduction of dioxygen indicating that the enzyme catalyzes the reduction to water, not hydrogen peroxide. As a further check on the stoichiometry of the catalyzed reduction an electroanalytical method was devised for the estimation of hydrogen peroxide in the solutions resulting from the enzyme-catalyzed reduction of dioxygen.

Graphite electrodes coated with the cobalt porphyrin described in reference (2) yield reversible voltammetric waves for the oxidation of hydrogen peroxide to dioxygen at pH values of 12 and above (2). Such a porphyrin-coated electrode was used in the form of a rotating disk to record the current potential curve shown in Figure 6 in a solution in which dioxygen had been reduced at a laccase coated electrode. The absence of a response near -0.15 volt attests to the absence of hydrogen peroxide. The reliability of the method was

Figure 6. Current potential curve for a rotating graphite disk electrode coated with a cobalt porphyrin (2) in the solution resulting from the reduction of dioxygen by (A) a laccase coated electrode; (B) a cobalt porphyrin coated electrode. The dioxygen was removed by bubbling the solution with argon and the pH was adjusted to 13 by addition of NaOH before the current-potential curves were recorded. The reduction of dioxygen was performed at 0 volt at a graphite disk electrode rotated at 100 rpm in 0.1 M $NaClO_4$ at pH 3.8 and saturated with O_2. In both cases 0.2 coulombs were consumed in the reduction of dioxygen. The final solution volume was 10 ml.


10 μA
A
B
Current
-0.2
0
0.2
0.4
E vs. SSCE, Volt

demonstrated by repeating the experiment with a solution in which the same amount of dioxygen had been reduced at an electrode coated with the same cobalt porphyrin instead of laccase. The reduction proceeds quantitatively to hydrogen peroxide under these conditions (2) and the current-potential curve labeled B in Figure 6 shows the clear anodic response for hydrogen peroxide oxidation. By adding known amount of hydrogen peroxide to the solution in which curve A was recorded it was estimated that no more than 5% of the dioxygen could have been reduced to hydrogen peroxide at the laccase coated electrode.

If the reduction of dioxygen is carried out at more negative potentials, some hydrogen peroxide is produced even at laccase-coated electrodes. This results from the uncatalyzed reduction of dioxygen to hydrogen peroxide at the graphite surface. Since we determined that laccase is not a good electrocatalyst for the reduction of hydrogen peroxide to water both products accumulate in the solution when dioxygen is reduced at potentials negative of ca. 0 volt.

The intercept of the line in Figure 5B provides the value of i_k which is the steady current that would flow if the concentration of dioxygen at the electrode surface were maintained equal to its value in the bulk of the solution during its reduction. It represents the maximum rate at which the dioxygen can be reduced for a given enzyme coating. The value of k obtained from this intercept and equation (2) is $1.5 \times 10^4\ M^{-1}\ s^{-1}$. In a solution saturated with O_2 (C_{O_2} - 1.2×10^{-3} M) this rate constant leads to a calculated enzyme turn-over rate of ca. 20 s^{-1}.

pH Dependence. To assess the pH dependence of the potential at which the reduction of dioxygen proceeds at laccase-coated electrodes the potential where the current reached 2.5 μA near the foot of cyclic voltammetric waves were recorded. (Half-wave potentials lose their special significance for electrode reactions controlled by the rates of antecedent chemical reactions.) The results, collected in Table 1, show essentially no pH dependence of the potential between pH 2.8 and 3.8 with a trend toward less positive potentials as the pH is increased to 4.7 and 5.5. This behavior contrasts with the uniform 60 mV per pH unit reported in reference (6b) between pH 3 and 6. The absolute values of the potentials for dioxygen reduction reported in reference (6) are also more positive at all pH values than those we observed.

The values of i_k obtained for laccase-coated rotating disk electrodes exhibited considerable dependence on the pH of the supporting electrolyte solutions. In Figure 7 the values of i_k measured between pH 2.4 and 5.5 are shown. The maximum catalytic rate is obtained between pH 3 and 4. It falls off more rapidly as the pH is lowered below 3 than it does when the pH is raised above 4. The decrease in i_k as the pH is lowered probably reflects denaturation of the immobilized

TABLE 1

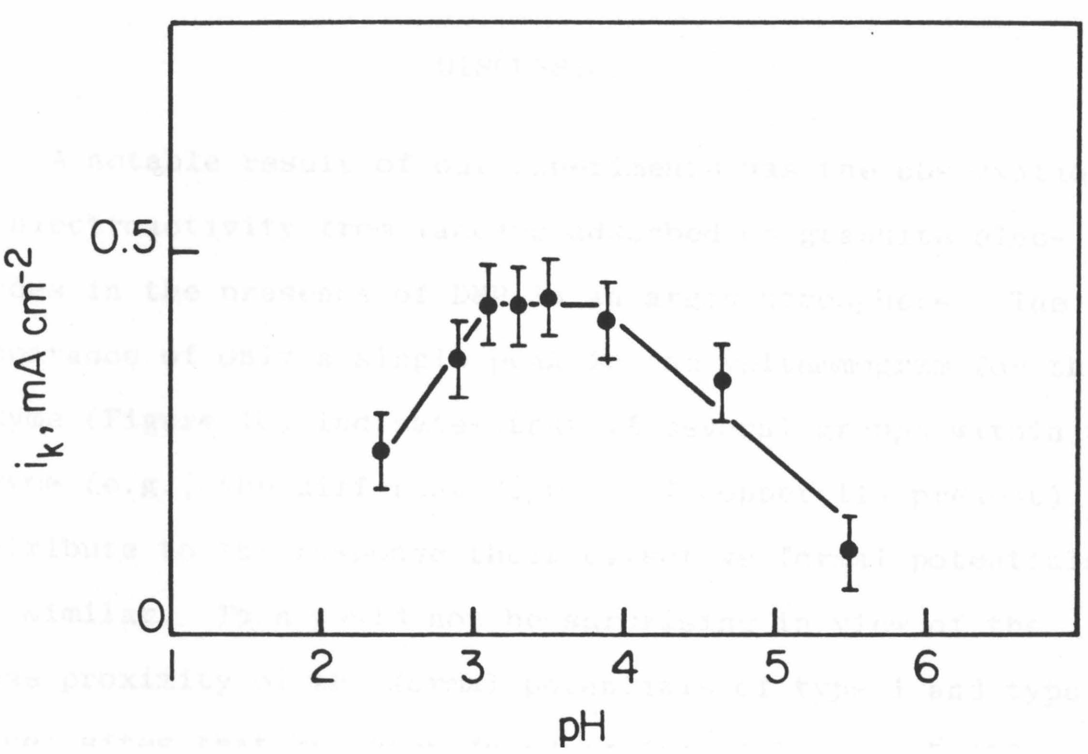
pH Dependence of the Potential where Dioxygen is Reduced at Laccase-Coated Graphite Electrodes[a,b]

pH	E vs. SSCE, mV
2.8	546
3.1	553
3.3	545
3.5	551
3.8	560
4.7	506
5.5	412

a. The potentials are the values at which the current reached 2.5 μA during the recording of a cyclic voltammogram at a scan rate of 10 mV s^{-1}.

b. Supporting electrolyte: 0.1 M $NaClO_4$ + the buffers given in the Experimental Section.

Figure 7. pH dependence of the kinetic currents obtained from the intercepts of Koutecky-Levich plots such as that in Figure 5B.


i_k, mA cm^{-2}
0.5
0
1
2
3
4
5
6
pH

enzyme as similar decreases in catalytic rates are also observed in homogeneous solutions of the enzyme at low pH where denaturation is observed (14). The decrease in rate at higher pH is also observed in homogeneous solution and has been attributed to possible morphological changes in the enzyme resulting from alteration in the coordination environment about the copper metal centers in the active site (15).

DISCUSSION

A notable result of our experiments was the observation of electroactivity from laccase adsorbed on graphite electrodes in the presence of DMP in an argon atmosphere. The appearance of only a single peak in the voltammogram for the enzyme (Figure 1C) indicates that if several groups within the enzyme (e.g., the different "types" of copper(II) present) contribute to the response their effective formal potentials are similar. This would not be surprising in view of the close proximity of the formal potentials of type 1 and type 3 copper sites that has been found in fungal laccase B (16).

Our observation of electrocatalytic activity of the immobilized enzyme is in accord with previous reports (6). However, uniformly more positive potentials are reported in reference (6b). For example, values of about 0.7 to 0.75 volt vs. SSCE are reported at pH 3 (6b) while we observed a potential near 0.55 volt at this pH. The experiments of reference (6b) employed electrodes coated with carbon black having an area of 220 m^2 gr^{-1} and the correspondingly much smaller current densities would be expected to lead to more positive operating potentials for these electrodes. The smaller current densities could also have influenced the apparent pH dependences of the operating potentials. However, the small and somewhat erratic pH dependence that we observed at the lower pH values suggest that the enzyme behavior is qualitatively different when it is adsorbed on low area pyrolytic graphite instead of high area carbon black.

The potential where the catalyzed reduction of dioxygen proceeds at pH 5.5 is ca. 90 mV more positive than that where the electrochemical response from the enzyme itself appears (Figures 1C and 2). Electrocatalytic reduction of substrate at potentials less negative than the formal potential of the catalyst are expected in cases where the rate of the reaction between substrate and the reduced catalyst is high enough to maintain the concentration of reduced catalyst at the low level that is in nernstian equilibrium with the electrode (3, 12). The implication is that the reduced form of the enzyme reacts rapidly with dioxygen to initiate the catalytic cycle. The apparent formal potential of the redox process associated with the cyclic voltammetric response shown in Figure 1C, 0.41 volt vs SSCE, differs from the formal potential of the type 1 copper center in laccase A as evaluated from thin layer spectroelectrochemical experiments (0.54 volt) (8). Nevertheless, considering the many factors that can contribute to differences in effective formal potentials of dissolved and immobilized enzymes (17), the reversible waves in Figure 1C could arise from the reduction and re-oxidation of the metal centers in the active site of the enzyme. The potential where the laccase response appeared in solutions of DMP was reproducible within a range of ca. ± 50 mV from coating to coating and did not exhibit any clear pH dependence between pH 3.7 and 5.6. However, because of the unknown function of the DMP molecules that are required to obtain the enzyme response in the absence of oxygen, it would be unwise at this stage to assign the reversible couple to any particular redox process centered in the enzyme.

Although laccase-coated graphite electrodes achieve the reduction of dioxygen at unusually positive potentials, the maximum sustainable currents are not so impressive. The rate constant obtained from the intercept of the Koutecky-Levich plot in Figure 5B, $1.5 \times 10^4\ M^{-1}\ s^{-1}$, is over one hundred times smaller than the measured constant governing the oxidation of laccase by dioxygen in homogeneous solution (5). The difference could arise both from loss of laccase from the electrode surface between the time it is adsorbed and the time of the kinetic measurements and from denaturation of a portion of the laccase remaining on the surface. The latter could also be responsible for the faster decay of the dioxygen reduction current in cyclic than in steady state rotating disk voltammetric experiments if the rate of denaturation were increased by the changes in the electric field experienced by the adsorbed enzyme. Improvement in the catalytic performance of laccase-coated electrodes might be anticipated from alternate methods of attachment that could provide longer-lived coatings containing more enzyme per cm^2. Polymeric or polyelectrolyte coatings in which the enzyme is incorporated could prove to be particularly useful for these purposes (18). They seem worthy of experimental testing.

CONCLUSIONS

Fungal laccase A adsorbs irreversibly on the surface of pyrolytic graphite electrodes where it serves as a catalyst for the electroreduction of dioxygen. The reduction proceeds quantitatively to water at more positive potentials than 0 volt vs. SSCE. The catalytic activity of the enzyme is greatest between pH 3 and 4. A lower limit of $1.5 \times 10^4\ M^{-1}\ s^{-1}$ was estimated for the rate constant governing the reaction between the adsorbed laccase and dioxygen. Conditions were found where a reversible electrochemical response from laccase A immobilized on electrode surface could be observed in the absence of dioxygen.

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CHAPTER IV

PROGNOSIS

Probably the most direct conclusion to be extracted from the present investigations would be that copper-containing molecules have the potential to be promising electrocatalysts for dioxygen reduction. The example shown in Chapter III has a superior ability in overcoming the overvoltage for the direct reduction of molecular oxygen to water. Accelerating the reduction rate with a reasonable stability may be one of the most important future research efforts to be added in developing laccase as an electrocatalyst. This may be possible if appropriately modified electrodes, which can incorporate the laccase firmly, are used along with highly mobile redox couples to transfer electrons from the electrode surface to the active sites of the enzyme. Indeed, a successful case has been observed recently by Buttry and Anson (1) with a Nafion coated electrode using a cobalt porphyrin with $Ru(NH_3)_6^{3+/2+}$ as an electron shuttle. The electrocatalytic system was shown to be stable for a long time. Preliminary experiments adopting this approach (2) showed that the $Mo(CN)_8^{4-/3-}$ couple acts as a good electron shuttle between immobilized laccase and the electrode surface.

The large size of the laccase molecule may be one of the most important factors to be considered in the developments of the enzyme as an electrocatalyst. The large size of the enzyme limits the use of common polymers as the matrix material to incorporate the bulky molecule or to physically entrap it. The use of a matrix material with larger pores (3) may be worth examining in this regard. Entrapping methods which employ complex formation between polymeric materials (4) may be also worth studying.

An alternative approach may be to use only part of the enzyme, as it may be possible to carve the active sites out. For some cases (5), carving out active sites and retaining enzymic activity has been successful. In this context, it may be valuable to use multicopper-containing biomimetic molecules. This would be equally worth pursuing as is the carving method, because, in several cases (6), successful biomimetic molecules containing metal ions have been prepared to closely imitate the original enzymes. In any case, further developments of laccase as an electrocatalyst for dioxygen reduction will remain a challenging problem for electrochemists who strive to solve the energy problems that face the world. Certainly, cooperation with chemists in other fields will be required to accelerate the developments.

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APPENDIX I

In Chapter II of this thesis, the new electrochemical technique of potential step chronocoulometry with a rotating disk electrode was applied to the measurement of the quantity of a Cu(I) complex accumulated on the electrode surface. Because only a brief discussion of the method was presented, a detailed description of the technique is given in this section.

Chronocoulometry (1, 2) has been one of the most popular electrochemical techniques among electrochemists because it offers important electrochemical advantages: (1) the measured signal often grows with time, and hence the later parts of the transient, which are most accessible experimentally and are least distorted by nonideal potential rise, offer better signal-to-noise ratios that the early time results. (2) The act of integration smooths random noise on the current transients, and hence the chronocoulometric records are inherently cleaner. (3) Contributions to the total charge from the double-layer charging and from electrode reactions of adsorbed species can be distinguished from those due to diffusing electroreactants. This latter advantage of chronocoulometry is especially valuable for the study of surface processes.

The charge integrated in the single step chronocoulometric mode contains contributions from three sources:

$$Q = Q_{d.l.} + Q_{ads} + Q_{diff} \tag{1}$$

where $Q_{d.l.}$ is the capacitive charge, Q_{ads} (= $nFA\Gamma$) the faradaic component arising from the reduction (or oxidation) of an adsorbed electroactive species, and Q_{diff} is the diffusional component of the charge. With stationary electrodes, Q_{diff} is given by

$$Q_{diff} = 2nFA\sqrt{\frac{D_o \cdot t}{\Pi}}\, C_o^b$$

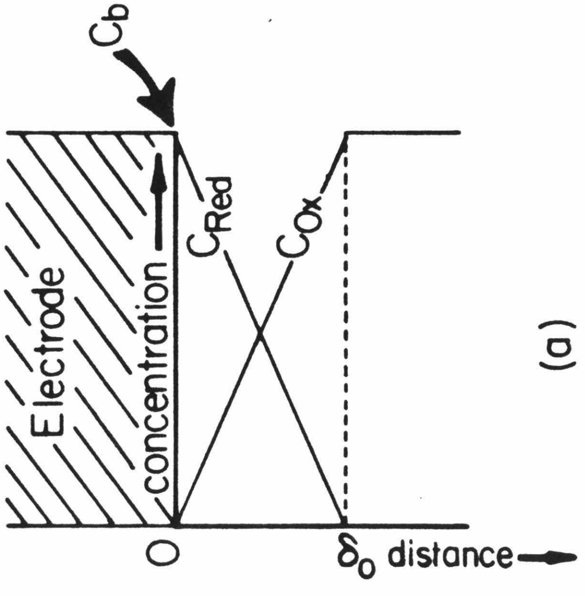
where n is the number of electrons transferred, F the Faraday, A the area of the electrode, D_o the diffusion coefficient of the electroactive species, t the time and C_o^b the bulk concentration of the electroactive species.

On the other hand, the rotating disk electrode is a novel electrochemical system where the flux of electroreactant becomes constant at a specific rotation rate. The hydrodynamic and convective-diffusion equations have been rigorously solved (3, 4). The diffusion layer (Levich layer) is given by

$$\delta_o = 1.61\ D_o^{1/3} \omega^{-1/2} \nu^{1/6} \tag{2}$$

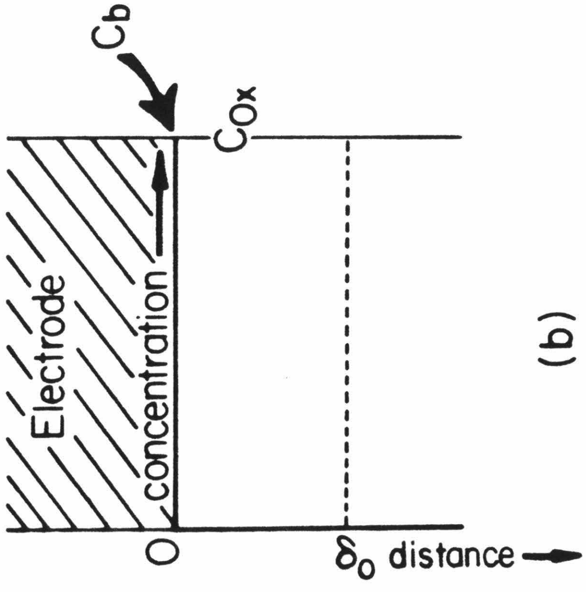
where δ_o is the angular frequency of rotation in sec^{-1}, and ν the kinematic viscosity of the solvent (0.01 cm^2/sec in water). The total quantity of reduced (or oxidized) species within the Levich layer (see Figure 1) will be approximately $(\frac{1}{2}) \cdot \delta_o \cdot A \cdot C_o^b$ (mole) at the potential where the current reaches the limiting plateau.

Figure 1. Schematic profile for concentration profile at rotating disk electrode (A) at the potential where the current reaches the limiting plateau (before the potential step), (B) at the potential where the current has decreased to background levels (after the potential step). Initial solution is assumed to contain the oxidized species only.



C_b
Electrode
concentration
C_{Red}
C_{Ox}
0
δ_0 distance

(a)



C_b
C_{Ox}
Electrode
concentration
0
δ_0 distance

(b)

When the potential of rotating disk electrode is stepped from the value where the reduction of electroactive species occurs to the value where the reoxidation of the reduced species does, the charge accumulated in the chronocoulometry will still be given by equation (1). Q_{diff}, however, will be only due to the contribution from the reduced electroactive species within the Levich layer because the rest of the electrogenerated reduced species is stirred into the large volume of the solution containing only the oxidized species and is thus diluted to a negligibly small concentration. It will be given approximately by

$$Q_{diff} = \tfrac{1}{2}\cdot n\cdot F\cdot A\cdot \delta_o\cdot C_o^b \tag{3}$$

The experimental tests for the method are presented in Table 1. $Ru(NH_3)_6^{3+}$ was chosen as an example because it does not adsorb on the electrode surface, namely $Q_{ads} = 0$. The $Q_{d.l.}$ obtained from the identical experiments with the blank solution was 52.3 μc/cm^2. The value of Q_{diff} calculated from equation (3) is 53.4 μc/cm^2 (the diffusion coefficient used in equation (2) was 7.19 x 10^{-6} cm^2/sec). The lower values observed in Table 1 indicate that most of the $Ru(NH_3)_6^{2+}$ within the Levich layer is reoxidized at the rotating disk electrode surface but that some of the $Ru(NH_3)_6^{2+}$ escapes re-oxidation and diffuses out into the bulk solution.

Potential step hydrodynamic chronocoulometry, taking advantage of the certain merits of both chronocoulometry and

TABLE I

Potential Step Hydrodynamic Chronocoulometry[a]

with 1 mM $Ru(NH_3)_6^{3+}$ in 0.1 M NaCl Solution at pH = 5.6

Equilibration time at -0.5 V (sec)		Q_{diff} ($\mu c/cm^2$)
5		42.9
10		43.1
20		45.3
50		42.5
100		44.7
	Avg.	43.7

[a] Basal plane pyrolytic graphite electrode was rotated at 1600 rpm; potential was stepped from -0.5 V to + 0.3 V.

the rotating disk electrode system, may be applied to the measurements of electroreactants confined on electrode surfaces directly or indirectly. For example, the quantitative assay of the incorporated electroactive species within polymer coated electrodes can be made in a straightforward manner by the method described herein.

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APPENDIX II

In Chapter II of this thesis, some comments were made on the origin of the mutually discrepant previous estimates of the rate constant for self-exchange between $Cu(phen)_2^{++}$ and $Cu(phen)_2^{+}$ in homogeneous solution. In this section, an approximate method will be applied to calculate the rate of electron exchange between Cu(I) and Cu(II) in their 1,10-phenanthroline and 2,2'-bipyridine complexes in aqueous media. The method may prove of general utility in determining the self-exchange rate constant for the redox couple of which the coordination numbers depend on the oxidation states.

It is now standard practice to use the Marcus relations (1) to correlate the rates of electron self-exchange and cross-reactions among redox reagents (2). The correlation takes its simplest form in cases where a redox couple makes the same contribution to the reorganization energy of every cross-reaction in which it engages (1). Frese (3) has recently discussed a number of experimental systems involving reactants exhibiting very small inner-space reorganization energies that appear to correspond closely to this case. However, with redox couples comprised of structurally dissimilar oxidized and reduced halves and correspondingly large inner-sphere reorganization energies, this simplifying assumption may not hold for cross-reactions. In the course of electrochemical kinetic experiments involving 1-10-phenanthroline (= phen) complexes of Cu(II) and Cu(I) (8), we required an estimate of the rate constant for self-exchange for this couple. Previous estimates for this rate constant in the literature (4, 5) were obtained by application of the Marcus correlation to measured cross-reaction rate constants under the simplifying assumption about reorganization energies mentioned above. The oxidation of tetrahedral $\mathrm{Cu(phen)_2^+}$ is believed to involve both a change in coordination geometry (5) and an expansion of the coordination sphere to produce a Cu(II) species such as $\mathrm{Cu(phen)_2OH_2^{2+}}$ (6, 7) in aqueous media in the absence of good ligands for Cu(II). For this reason the derivation of self-exchange reorganization energies from cross-exchange reactions and the simplifying assumption seemed questionable. In this note the previous experimental data are re-analyzed without making this assumption to obtain a new estimate of the rate constant for self-exchange. The resulting value is in reasonably good agreement with a recent estimate for this constant obtained from electrochemical experiments (8).

RESULTS AND DISCUSSION

The kinetics of cross-reaction (1) have been measured by Holwerda et al. (4) (edta = ethylenediamminetetraacetate):

$$\begin{aligned} &\mathrm{Cu(phen)_2^{+} + Co^{III}(edta)^{-} + H_2O \rightarrow} \\ &\mathrm{Cu(phen)_2(OH_2)^{2+} + Co^{II}(edta)^{2-}} \end{aligned} \qquad (1)$$

From their data the overall activation energy for the cross-reaction and the corresponding reorganization energy, λ, were calculated using the relevant equations of Sutin (2) as given by Frese (3). The resulting value of λ is listed in Row 1 of Table 1. The reorganization energy for self-exchange reactions between $\mathrm{Co^{III}(edta)^{-}}$ and $\mathrm{Co^{II}(edta)^{2-}}$, calculated in the same way from the known activation energy for the self-exchange (3, 4) is given in Row 3 of Table 2. The $\mathrm{Co^{III}(edta)^{-}/Co^{II}(edta)^{2-}}$ couple is among those which have been shown to adhere to the simplest form of the Marcus correlation in several cross reactions (1b). Accordingly, the contribution made by $\mathrm{Co^{III}(edta)^{-}}$ to the λ value for reaction (1) was taken as one-half of the λ value for the $\mathrm{Co^{III}/Co^{II}}$ self-exchange reaction. Subtracting half of the latter value from λ for reaction (1) gives the value of $\lambda_{R \rightarrow O}$, 0.48 eV, listed in Row 1 of Table 1. The value of 0.48 eV represents the contribution of $\mathrm{Cu(phen)_2^{+}}$ to the reorganization energy in reaction (1) (and, of course, the contribution of $\mathrm{Cu(phen)_2(OH_2)_2^{2+}}$ to the reorganization energy for the reverse of reaction (1)).

The reorganization energy associated with the reduction of $\mathrm{Cu(phen)_2(OH_2)^{2+}}$ was estimated in a similar fashion from the kinetic data of Augustin and Yandell for reaction (2)

TABLE 1

Rate Constants and Activation Parameters

Row Number	Redox Pairs	Ref.	ΔG^o,[a] eV	E_f,[b] mV	$\Delta G^{\ddagger}$,[c] eV	$10^2 W^r$,[d] eV	σ,[e] Å	λ,[f] eV	$\lambda_{O\rightarrow R}$,[g] eV	$\lambda_{R\rightarrow O}$,[h] eV	k,[j] $M^{-1}s^{-1}$
1	$Co^{III}(edta)^-$/ $cu(phen)_2^+$	4	-0.203		0.543	-0.428	11.6	2.60	2.12	0.48	3.12×10^2
2	$Cu(phen)_2^{2+}(OH_2)$/ Cyt $c(II)^{6.5+}$	5	+0.087		0.632	+2.91	23.7	2.26	1.28	0.98	2.72×10^1
3	$Co^{III}(edta)^-$/ $Co^{II}(edta)^{2-}$	4,11	0	377[k]	1.07	+1.23	9.4	4.24	2.12	2.12	3.20×10^{-7}
4	Cyt $c(III)^{7.5+}$/ Cyt $c(II)^{6.5+}$	9	0	261[l]	0.550	+6.06	33.2	1.96	0.98	0.98	3.50×10^2
5	$Cu(phen)_2(OH_2)^{2+}$/ $Cu(phen)_2^+$		0	174[m]	0.451	+1.07	14.0	1.76[n]	0.88	0.88	1.55×10^4 [n]
6	$Co^{III}(edta)^-$/ $Cu(bpy)_2^+$	4	-0.257		0.547	-0.455	11.2	2.72	2.12	0.60	2.59×10^2
7	$Cu(bpy)_2(OH_2)_2^{2+}$/ Cyt $c(II)^{6.5+}$	5	+0.141		0.660	+2.98	23.4	2.26	1.28	0.98	1.39×10^1
8	$Cu(bpy)_2(OH_2)_2^{2+}$/ $Cu(bpy)_2^+$		0	120[m]	0.482	+1.16	13.3	1.88[n]	0.94	0.94	4.41×10^3 [n]

TABLE 1 (continued)

a. Standard free energy change of the reaction between the redox pair

b. Formal potential of the redox couple vs. NHE

c. Activation free energy for the same reaction

d. Work expended in bringing the reactant redox pair together. Calculated from equations 5 and 6 of reference 2, as appropriate

e. Sum of the radii of the redox pair

f. Total reorganization energy for reaction, calculated from equation 16 of reference 3

g. Reorganization energy contribution by the oxidized reactant in the redox pair

h. Reorganization energy contribution by the reduced reactant in the redox pair

j. Rate constants for the reaction between the redox pair. Measured values are given except for those labeled (n)

k. Reference 12

l. Reference 13

m. Reference 7

TABLE 1 (continued)

n. Values calculated from the sum of $\lambda_{O\rightarrow R}$ and $\lambda_{R\rightarrow O}$ for the two corresponding cross-reactions.

$$\mathrm{Cyt\ c(II)} + \mathrm{Cu(phen)_2(OH_2)^{2+}} \rightarrow \mathrm{Cyt\ c(III)} + \mathrm{Cu(phen)_2^{+}} + \mathrm{H_2O} \qquad (2)$$

The required activation energy for self-exchange between Cyt c(III) and Cyt c(II), listed in Row 4 of Table 1, was calculated from kinetic data given in reference (9). The cytochrome c couple is also one which has been shown to adhere to the simple form of the Marcus correlation (9) so that the contribution of Cyt c(II) to the reorganization energy involved in reaction (2) was taken as half of that for its self-exchange reaction. The resulting calculated contribution of $\mathrm{Cu(phen)_2(OH_2)^{2+}}$ to the reorganization energy of reaction (2) (or of $\mathrm{Cu(phen)_2^{+}}$ to the reverse of reaction (2)) is $\lambda_{O \rightarrow R} = 1.28$ eV (Table 1, Row 2). The much smaller reorganization energy obtained for the oxidation of $\mathrm{Cu(phen)_2^{+}}$ by $\mathrm{Co^{III}(edta)^{-}}$ than for the reduction of $\mathrm{Cu(phen)_2(OH_2)^{2+}}$ by Cyt c(II) is compatible with the conversion of the coordination geometry about the Cu center from tetrahedral in the Cu(I) complex to distorted trigonal bipyramidal or octahedral in the Cu(II) complex (5, 6, 7).

It seems reasonable, although admittedly ad hoc at present, to take the sum of $\lambda_{R \rightarrow O}$ and $\lambda_{O \rightarrow R}$ for the copper complexes as the total reorganization energy for the self-exchange reaction between $\mathrm{Cu(phen)_2(OH_2)^{2+}}$ and $\mathrm{Cu(phen)_2^{+}}$. This sum was therefore used to evaluate the overall activation energy and rate constant listed in Row 5 of Table 1. The resulting calculated value of the rate constant for self-exchange, k = 1.55 x 10^4 M^{-1} s^{-1}, differs from both previous estimates (5 x 10^7 M^{-1} s^{-1} (4) and 50 M^{-1} s^{-1} (5)) that were obtained by assuming that the two halves of the couple make the same contribution to the overall reorganization energy of both self-exchange and cross-reactions. (Corresponding recalculation of kinetic data for the complexes with 2,2'-bipyridine (=bpy) (4, 5, 10) are also listed in Table 1, Rows 5, 7, 8.) We believe that the calculated values of the self-exchange rate constant in Table 1 is probably more reliable because recent rotating disk electrochemical measurements (8) provided an estimate of this rate constant for the Cu (II, I) couple of ca. 10^5 M^{-1} s^{-1}. Thus, these Cu(II)/Cu(I) couples appear to represent examples of a class of redox reactants that make distinctly different contributions to the reorganization energies of the cross-reactions in which they participate. This class seems likely to encompass additional redox couples and the procedure outlined above may prove of general utility in dealing with such systems.

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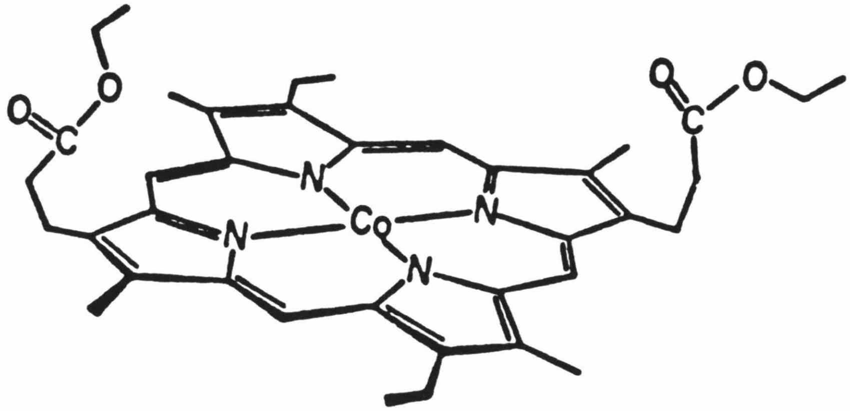
APPENDIX III

The detection of hydrogen peroxide has been one of the major problems encountered in catalytic studies of the electroreduction of molecular oxygen (1-3). The ring-disk electrode method is hampered by the poisoning effects of oxidation of hydrogen peroxide. The usual method is to compare the slopes of the Koutecky-Levich plots to those of two-electron and four-electron processes in order to determine the reduction products. In this section, a new electroanalytical method of detecting hydrogen peroxide will be described. The method was utilized to analyze the products in the reduction of dioxygen catalyzed by reduced fungal laccase A in Chapter III of this thesis.

The method is based on two well known facts: (1) cobalt porphyrins (Figure 1) adsorbed on graphite electrode surfaces yield reversible voltammetric waves for the oxidation of hydrogen peroxide to dioxygen at high pH (> 12) (2). And (2) the electroreduction of dioxygen at rotating disk electrodes in the solution saturated with oxygen generates the products at substantially measurable quantities that can be directly quantified by measuring the ejected charge at the electrode with a coulometer.

After the pH of the solution containing the products from the electroreduction of dioxygen is raised (to > 12) by

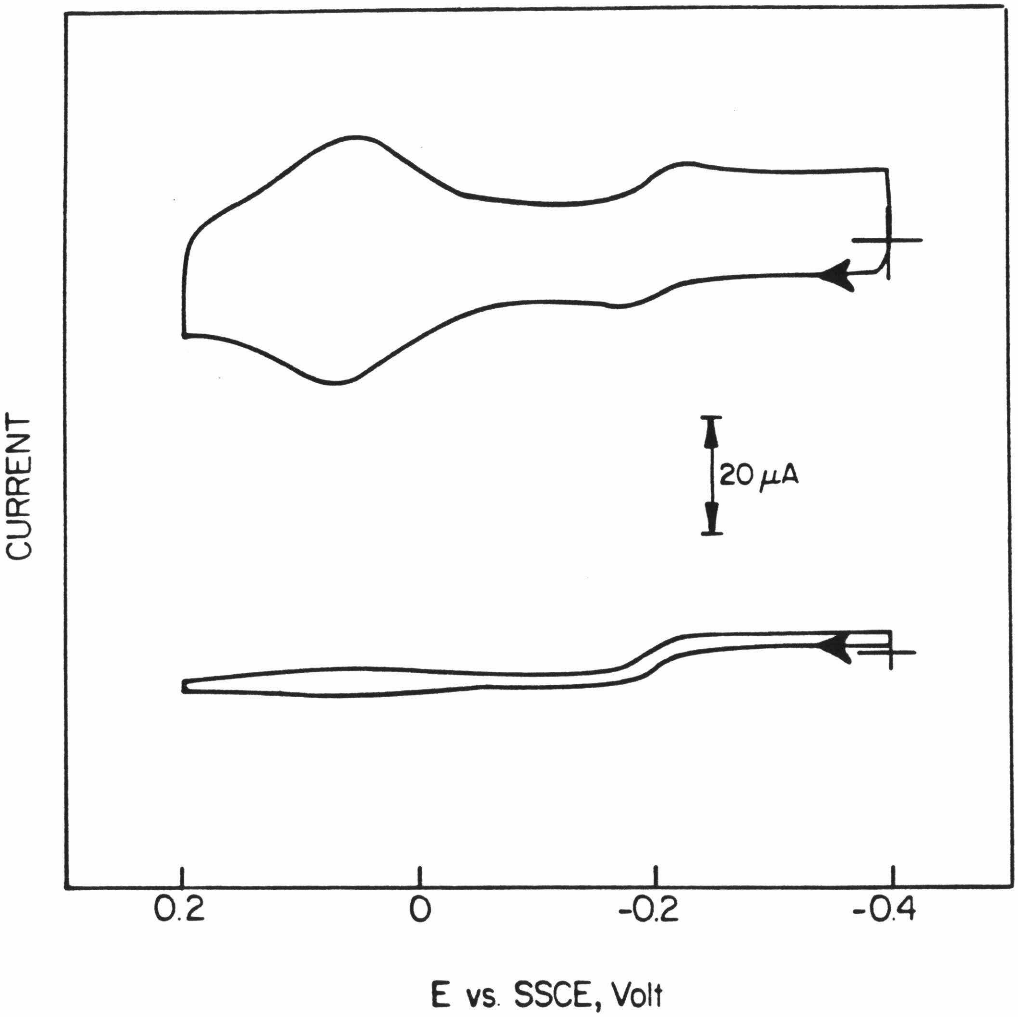
Figure 1. The cobalt porphyrin catalyst employed in the electroanalytical method of detecting hydrogen peroxide.


O
C
O
N
N
Co
N
N
O
C
O

adding sodium hydroxide pellets and the solution is deaerated with pre-purified argon, just one voltammetric response of the rotating disk electrode freshly coated with the cobalt porphyrin under oxygen free atmosphere will give the information about whether hydrogen peroxide was formed or not in the reaction. The reliability of the method was tested by doing the experiments with a solution in which a known amount of dioxygen had been reduced, at an electrode coated with the cobalt porphyrin, to yield 0.1 mM H_2O_2. The results are shown in Figure 2. The immobilization of the cobalt porphyrin on electrode surfaces was checked by the cobalt wave at +0.1 V (2). Limiting current at low rotation rate (100 rpm) could lead to a quantitative estimate of hydrogen peroxide added.

Knowing the chemical fact that the reduction of molecular oxygen produces hydrogen peroxide and/or water, the method should give a clear analysis of the products of dioxygen reduction.

Figure 2. Cyclic voltammogram (upper; 100 mv s^{-1}) and rotating disk voltammogram (bottom; 10 mv s^{-1}) for the reduction products with the cobalt porphyrin coated electrode under an argon atmosphere. The reduction of molecular oxygen was conducted by rotating the cobalt porphyrin coated electrode at 0 V (vs. SSCE) with a rotation rate of 100 rpm for the pH 3.8 electrolyte solution (0.1 M $NaClO_4$ + 0.02 M acetate buffer) under oxygen atmosphere (total ejected charge; $\frac{2 \times 10^{-1} \text{ coul}}{10 \text{ ml}}$). Final solution for the analysis was adjusted to pH 13 by adding one sodium hydroxide pellet per 10 ml of electrolyte solution.


CURRENT
20 μA
0.2
0
-0.2
-0.4
E vs. SSCE, Volt

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