inhibitors of bovine plasma and pig kidney amine oxidase [3, 4].

Cu(II) complexes of 1,10-phenanthroline (phen) and 2,2'-bipyridine (bipy) strongly inhibited both amine oxidases. Lysine, tyrosine, and salicylate complexes, as well as Cu^{2+}(aq.), inhibited the plasma but not the kidney amine oxidase. No inhibition by the free ligands, by $Cu(EDTA)^{2-}$, or bovine erythrocyte SOD was observed. $Cu(phen)_2(NO_3)_2$, Cu^{2+}(aq.), and $Cu(salicylate)_2$ displayed linear noncompetitive inhibition against amine substrates at saturating O_2 concentrations with either pig kidney or beef plasma amine oxidase. Since neither the free ligands nor Cu^{2+}(aq.) inhibit the kidney enzyme at concentrations where $Cu(phen)_2(NO_3)_2$ and $Cu(bipy)_2Cl_2$ are effective, the observed inhibitory action must be attributed to the complexes. Two plausible inhibition mechanisms are (1) dismutation of O_2^-, which is an intermediate in the reoxidation reaction; or (2) direct oxidation of E_{red} by the Cu(II) complexes. Steric requirements for the second mechanism should be less restrictive than for mechanism (1) as outer-sphere electron transfer is possible over substantial distances, but O_2^- dismutation is an inner-sphere process that requires the Cu(II) complex to penetrate to the O_2 reduction site. Thus if O_2^- is tightly bound within the dioxygen-reduction site, its accessibility to Cu(II) complexes of varying size and charge would be mechanistically important. On this basis the reactivity pattern we have observed can be rationalized: only those complexes with hydrophobic ligands and a net positive charge inhibited pig kidney amine oxidase, but the active site in the plasma enzyme may be much more accessible to external reagents.

N_3^- and SCN^- are Cu(II)-specific inhibitors of several amine oxidases [1, 5–7]. Ligand-to-metal charge-transfer bands are evident at ~365 nm ($\epsilon \sim$ 3,000 M^{-1} cm^{-1}) and ~400 nm ($\epsilon \sim 6{,}000$ M^{-1} cm^{-1}) when SCN^- and N_3^-, respectively, are added to beef plasma or pig kidney amine oxidase. The intensities and energies establish that the anions are equitorially coordinated. Changes in the Cu(II) ligand field, as judged by absorption, circular dichroism and EPR spectroscopy, are consistent with a simple ligand substitution reaction. Preliminary NMR evidence indicates that H_2O is the leaving group, as previously demonstrated for the pig plasma enzyme [6]. Depending on the amine concentration both anions are linear noncompetitive or linear uncompetitive inhibitors of beef plasma amine oxidase with O_2 saturating; under the same conditions these anions were strictly linear uncompetitive inhibitors of the pig kidney enzyme. By determining in detail the inhibition mechanism of anions and the Cu(II) complexes, we should be able to learn much about the role(s) of copper in the amine oxidases.

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E6

Activation of Dioxygen by Electron-transfer Reduction in the Presence of Protons or Transition Metal Complexes

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Electrochemical studies of molecular oxygen in aprotic and aqueous media confirm that the primary electron-transfer step for the reduction of O_2 is a one-electron process, which is followed by chemical disproportionation reactions to yield H_2O_2 under acidic conditions (an overall two-electron process) and OH^- under basic conditions (an overall four-electron process) [1]. Spectroscopic, electrochemical, and kinetic measurements have been used to characterize the reactions of O_2^-, $HO_2^\bullet$, H_2O_2, and HO_2^- in aprotic solvents. Superoxide ion (O_2^-) is a strong Bronsted base (*e.g.*, deprotonates butanol and water), an effective nucleophile (*e.g.*, reacts rapidly with alkyl halides and esters by an S_N2 mechanism), and a moderate one-electron reducing agent [2]. It rapidly degrades polyhalogenated hydrocarbons (CCl_4, $HCCl_3$, and DDT) to oxygenated products [3]. For substrates with oxidizable hydrogen atoms (hydrazines, hydroxylamines, and reduced flavins), O_2^- is an effective one-electron oxidizing agent [4]. Superoxide also readily oxygenates benzyl and dehydroascorbic acid [5], and couples with reduced paraquat to yield an oxygenating reagent [6]. Hence, a primary route for the biochemical activation of $^3O_2$ to an effective electron acceptor, oxidase, monooxygenase, and dioxygenase is an initial reduction to superoxide ion.

Reduction of O_2 in the presence of protons and transition metal ions yields highly reactive perhydroxyl and peroxide intermediates [7, 8].

$$O_2 + H^+ + e^- \xrightarrow[DMF]{+0.12\ V\ vs\ NHE} HO_2^\bullet \rightarrow 1/2\ H_2O_2 + 1/2\ O_2 \quad (1)$$

$$O_2 + Zn(II) + 2e^- \xrightarrow[Me_2SO]{-0.5\ V} ZnO_2 \quad (2)$$

Likewise, reduction of dioxygen in the presence of activating substrates (transition metal complexes, esters, and methyl viologen (MV^{2+})) results in a concerted two-electron process to yield reactive oxygenating agents and reaction mimics for oxygenases [6].

$$MV^{2+} + O_2 + 2e^- \xrightarrow[DMF]{-0.5\ V} MV^+(O_2^-) \quad (3)$$

The interactions of O_2 and its reduction products with iron-porphyrin,

$$Fe^{III}TPP^+ + O_2 + e^- \rightarrow Fe^{II}TPP(O_2) \xrightarrow{e^-} Fe^{III}TPP(O_2^{2-})^- \quad (4)$$

copper-bipyridine,

$$Cu^{II}(Bipy)_2^{2+} + O_2 + 2e^- \rightarrow Cu^{II}(Bipy)(O_2^{2-}) \quad (5)$$

and manganese-catechol complexes [9]

$$Mn^{IV}(DTBC)_3^{2-} + O_2 + OH^- \rightleftharpoons Mn^{III}(DTBSQ)_2\ (DTBC)(O_2^-)(OH)^{3-} \rightarrow Mn^{III}(DTBC)_2(O_2^-)\ (OH)^{3-} + DTBQ \quad (6)$$

yield a variety of reactive intermediates; the O_2 adducts are further activated by reduction. Several examples will be discussed of the *oxidation* of organic and inorganic substrates *via reductive* activation of O_2.

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E7

Cyanide and Methylisocyanide: Probes for Nitrogenase Reactivity

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Nitrogenase (N_2ase) is composed of two separately purified proteins, the molybdenum–iron (MoFe) protein and the iron (Fe) protein. Nitrogen fixation requires both proteins, a reductant, protons and MgATP. The Fe protein is generally accepted as a specific one-electron donor for the MoFe protein, which is believed to contain the substrate-reduction site. Besides N_2, N_2ase catalyzes the reduction of protons and a number of alternative substrates [*e.g.* 1], including the two six-electron substrates, cyanide and methylisocyanide, we have recently studied.

The rate-limiting step for N_2ase turnover occurs prior to substrate reduction. Thus, total electron flow through the enzyme should be essentially independent of the substrate being reduced. Although this appears true for N_2 fixation, H_2 evolution and C_2H_2 reduction [2], both CN^- [3] and CH_3NC dramatically inhibit the rate of total electron flow through N_2ase. Inhibition by both substrates is completely reversed by CO. Not only do CN^- and CH_3NC inhibit nitrogenase turnover, they also reduce the enzyme's efficiency by increasing the amount of MgATP hydrolyzed for each electron pair used to reduce substrate. These data are interpreted in terms of CN^- or CH_3NC binding to the MoFe protein in such a way as to prevent electron transfer to substrate. With nowhere to go, the electrons fall back to the Fe protein to complete a futile cycle.

Are the substrates N_2, HCN and CH_3NC reduced in one six-electron step or via a series of lesser reduced intermediates? Previously, we proposed that N_2 is reduced to ammonia via the two-electron intermediates N_2H_2 and N_2H_4 [4]. For the six-electron reductions of HCN to $CH_4 + NH_3$ and CH_3NC to $CH_4 + CH_3NH_2$, we have definitively identified the four-electron products, CH_3NH_2 (for HCN) and CH_3NHCH_3 (for CH_3NC), and suggest them as intermediates. The formation of two-electron reduced intermediates for both HCN and CH_3NC is suggested by the product ratio of NH_3-to-CH_4 (for HCN) and CH_3NH_2-to-CH_4 (for CH_3NC) being greater than one.

The data support mechanisms whereby the six-electron reduction of N_2, HCN and CH_3NC occur via a series of analogous two- and four-electron reduced intermediates. Thus, a common phenomenon is likely as an intimate part of the mechanisms of N_2, HCN and CH_3NC reduction. Although H_2 evolution is suggested as an obligatory part of the N_2-fixation mechanism, it is *not* required for either HCN or CH_3NC