both for the gel state and for the liquid-crystalline state (Figure 3). We are currently investigating DPPC mixture of low weight percent in our laboratory.

Conclusions

High Ps formation probabilities in lipid bilayers enable us to apply the positronium annihilation technique to study the chemical and physical properties of biomembranes. The correlations between membrane fluidity and the Ps annihilation characteristics, particularly those of I_3 % and τ_3, show that Ps atoms are able to distinguish between various microstructural properties at least within a DPPC bilayer, and they reveal the hydrocarbon-like interior. If we assume the comparison can be made between the observed K_{Ps} values in the DPPC bilayer and in the values of various solvents, then we can suggest that the location of the *p*-benzoquinone charge transfer molecule is near the backbone polar region in the interior of the bilayers. These observations thus suggest that Ps atoms are potentially sensitive nuclear probes to study the dynamic behavior of charge transport and microstructures within biomembranes.

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Registry No. DL-α-Dipalmitoylphosphatidylcholine, 2797-68-4; *p*-benzoquinone, 106-51-4; positronium, 12585-87-4.

Mechanisms of the Dismutation of Superoxide Catalyzed by the Copper(II) Phenanthroline Complex and of the Oxidation of the Copper(I) Phenanthroline Complex by Oxygen in Aqueous Solution

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Abstract: By using the technique of pulse radiolysis to generate O_2^-, it is demonstrated that copper 1,10-phenanthroline ($(\text{op})_2\text{Cu}^{2+}$) is capable of catalytically dismutating O_2^- with a "turnover" rate constant $k_{\text{cat.}} = (5.1 \pm 0.9) \times 10^8\ \text{M}^{-1}\ \text{s}^{-1}$. The rate constants of the reduction of $(\text{op})_2\text{Cu}^{2+}$ and of the oxidation of $(\text{op})_2\text{Cu}^+$ by O_2^- have been determined to be $(1.93 \pm 0.07) \times 10^9$ and $(2.95 \pm 0.3) \times 10^8\ \text{M}^{-1}\ \text{s}^{-1}$, respectively. The kinetic results of the oxidation of $(\text{op})_2\text{Cu}^+$ by molecular oxygen in aqueous solution are interpreted by a mechanism that proceeds via a superoxide intermediate, and the rate constant $k_{-10} = (5.0 \pm 0.3) \times 10^4\ \text{M}^{-1}\ \text{s}^{-1}$ has been determined. The rate constant of the oxidation of $(\text{op})_2\text{Cu}^+$ by H_2O_2 was measured to be $k_{22} = (937 \pm 20)\ \text{M}^{-1}\ \text{s}^{-1}$.

Enzymatic catalysis of superoxide dismutation has been a matter for investigation since the work of McCord and Fridovitch.[1] It has been suggested that the mechanism of the catalytic dismutation of O_2^- by bovine superoxide dismutase (SOD) involves alternate reduction and oxidation of copper ions:[2,3]

$$\text{enzyme-Cu}^{2+} + O_2^- \xrightarrow{k_1} \text{enzyme-Cu}^+ + O_2 \qquad (1)$$

$$\text{enzyme-Cu}^+ + O_2^- + 2\text{H}^+ \xrightarrow{k_2} \text{enzyme-Cu}^{2+} + H_2O_2 \qquad (2)$$

The "turnover" rate constant, $k_{\text{cat.}} = (2.4 \pm 0.3) \times 10^9\ \text{M}^{-1}\ \text{s}^{-1}$, is pH independent over the range 4.8–9.5.[4-6]

A large number of copper compounds have been tested for the rate at which they react in aqueous solution with O_2^- to catalyze its dismutation. The aquo complex and the copper chelates of some amino acids[7-9] and salicylates[10] exert almost the same catalytic activity as SOD.

Recently, it has been demonstrated that degradation of deoxyribonucleic acid (DNA) by 1,10-phenanthroline requires Cu(II), a reducing agent, and O_2. Other metal ions do not substitute for Cu(II), and the degradation of DNA is inhibited by metal ions that can form stable complexes with 1,10-phenanthroline as well as by chelators that can bind copper. The degradation of DNA is also inhibited by either SOD or catalase, suggesting that O_2^- and H_2O_2, respectively, are required for the breakdown of the DNA.[11-14]

In view of the role of $(\text{op})_2\text{Cu}^{2+}$ in the degradation of DNA, and the possible role of O_2^- in this process, we have decided to study the catalytic effect of $(\text{op})_2\text{Cu}^{2+}$ upon the disproportionation of O_2^- and the kinetics and the reaction mechanism of the oxidation of $(\text{op})_2\text{Cu}^+$ by molecular oxygen in the presence and in the absence of H_2O_2.

Experimental

Pulse radiolysis experiments were carried out on a Varian 7715 linear accelerator. The pulse duration ranged from 0.1 to 1.5 μs with a 200-mA current of 5 MeV electrons. Either a 200W Xe–Hg or a 150W Xenon arc were used as the analytical light source. We used 2.0 or 4.0 cm long irradiation optical cells with one or three light passes. Appropriate light filters were used to eliminate any scattered light. The detection system included a Bausch & Lomb grating monochromator Model D330/D331

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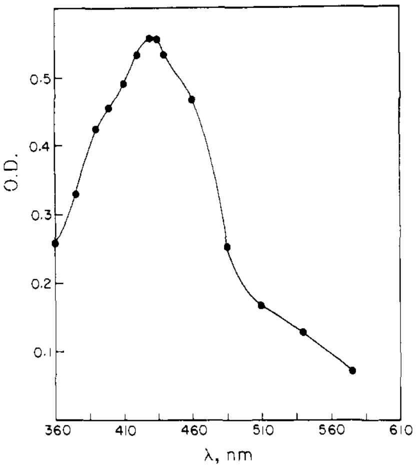

Figure 1. Spectrum of $(op)_2Cu^+$. The spectrum was taken in an oxygenated solution of 0.05 M sodium formate at pH 7 containing 150 μM $(op)_2Cu^{2+}$. The optical path was 12.3 cm, and the initial concentration of O_2^- was (6.1 ± 0.6) μM.

Mk.II and an IP28 photomultiplier. The signal was transferred through either a Biomation 8100 or an analog to digital converter to a Nova 1200 minicomputer, which operated the whole pulse radiolysis system.

All solutions were prepared in distilled water, which was further purified by a Milli-Q reagent grade water system (Millipore Corp., Bedford, Mass.). All chemicals employed were of analytical grade and were used without further purification: 1,10-phenanthroline hydrate (B.D.H.), cupric sulfate, monosodium and disodium phosphate (Mallinckrodt Chem. Works), sodium formate (Matheson, Coleman & Bell), and bovine erythrocuprein (Diagnostic Data Int.).

Unless otherwise stated, all solutions contained 0.05 M sodium formate plus 1 mM phosphate buffer (pH 7) and were saturated either by air or by oxygen. Kinetic studies of formation and decay of $(op)_2Cu^+$ were followed at λ 435 nm. We were not able to follow the decay of O_2^- at λ 250 nm, since the absorption of the complex in this region is too high.

The total concentration of O_2^- in the cell was evaluated with the use of potassium ferrocyanide dosimetry.[15] The yield of O_2^- in oxygenated formate solution was assumed to be 6.05.[16] The concentration of the radicals in the cell was 1.0–14.5 μM for 0.1–1.5-μs pulse duration, respectively.

Results and Discussion

In the irradiation of aqueous solutions containing formate ion and oxygen, the following reactions occur:

$$H_2O \rightsquigarrow e^-_{aq}, OH, H, H_2O_2, OH^-, H_3O^+ \tag{3}$$

$$e^-_{aq} + O_2 \rightarrow O_2^- \qquad k_4 = 2 \times 10^{10}\ M^{-1}\ s^{-1}\ {}^{17} \tag{4}$$

$$H + O_2 \rightarrow HO_2 \qquad k_5 = 2 \times 10^{10}\ M^{-1}\ s^{-1}\ {}^{18} \tag{5}$$

$$\begin{gathered} OH + HCO_2^- \rightarrow H_2O + CO_2^- \\ k_6 = 3 \times 10^9\ M^{-1}\ s^{-1}\ {}^{19} \end{gathered} \tag{6}$$

$$CO_2^- + O_2 \rightarrow CO_2 + O_2^- \qquad k_7 = 2.4 \times 10^9\ M^{-1}\ s^{-1}\ {}^{20} \tag{7}$$

$$HO_2 \rightleftharpoons H^+ + O_2^- \qquad K = (2.05 \pm 0.39) \times 10^{-5}\ M^{16} \tag{8}$$

When $(op)_2Cu^{2+}$ is also present, it may compete with oxygen for e^-_{aq} and CO_2^- and with formate ions for OH radicals. At sufficiently high concentration of formate and oxygen relative to $(op)_2Cu^{2+}$, only two processes take place:

$$(op)_2Cu^{2+} + CO_2^- \rightarrow (op)_2Cu^+ + CO_2 \tag{9}$$

$$(op)_2Cu^{2+} + O_2^- \rightarrow (op)_2Cu^+ + O_2 \tag{10}$$

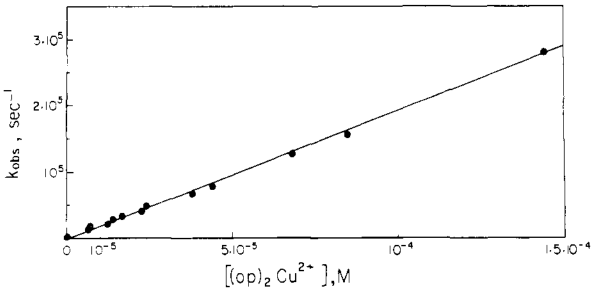

Figure 2. Formation rate constant of $(op)_2Cu^+$ as a function of $[(op)_2Cu^{2+}]$. All solutions contained 0.05 M sodium formate at pH 7 and were air saturated. The pulse duration was 0.5 μs.

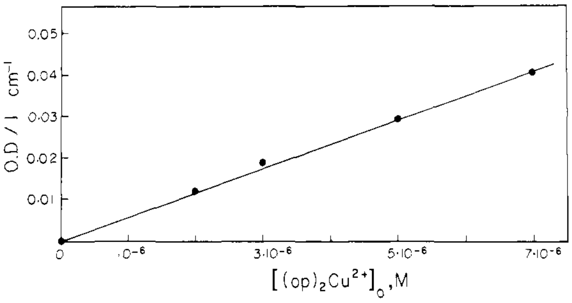

Figure 3. The absorption of $(op)_2Cu^+$ at the end of the pulse as a function of the initial concentration of $(op)_2Cu^{2+}$. All solutions contained 0.05 M sodium formate at pH 7 and were air saturated. The pulse duration was 1.5 μs.

The Absorption Spectrum of $(op)_2Cu^+$. Figure 1 gives the optical absorption of $(op)_2Cu^+$ obtained in a solution saturated with oxygen, where $(op)_2Cu^{2+}$ is in excess relative to O_2^- and CO_2^-. The same results were obtained in solutions saturated with helium, where CO_2^- and e^-_{aq} reduce the copper complex. The $(op)_2Cu^+$ is stable for a couple of minutes in the absence of oxygen. The absorption maxima is in the region λ_{max} 430–435 nm with an extinction coefficient of (7400 ± 800) $M^{-1}\ cm^{-1}$ at λ 435 nm. These observations are in agreement with those obtained in aqueous acetonitrile (2.5 M)[21] and in 40% methyl carbitol solution.[22]

The Kinetics of the Formation of $(op)_2Cu^+$. We have determined the rate constant of reaction 9 by following the absorption change at 435 nm in solutions containing formate ion and saturated with helium. We found that at high concentrations of $(op)_2Cu^{2+}$ relative to CO_2^-, the formation of $(op)_2Cu^+$ is pseudo first order. The second-order rate constant $k_9 = (1.2 \pm 0.05) \times 10^9\ M^{-1}\ s^{-1}$ was obtained.

When the solutions are oxygen saturated, CO_2^- is converted into O_2^- and therefore only reaction 10 takes place. Rate eq 11 is obtained. At high $[(op)_2Cu^{2+}]/[O_2^-]$ ratios we observed

$$d(O_2^-)/dt = d[(op)_2Cu^+]/dt = k_{10}[(op)_2Cu^{2+}][O_2^-] \tag{11}$$

pseudo-first-order kinetics where $k_{obsd} = k_{10}[(op)_2Cu^{2+}]$. The second-order rate constant $k_{10} = (1.93 \pm 0.07) \times 10^9\ M^{-1}\ s^{-1}$ was obtained by plotting k_{obsd} vs. $(op)_2Cu^{2+}$ concentration (Figure 2).

At low $(op)_2Cu^{2+}$ concentrations, where $[(op)_2Cu^{2+}] < [O_2^-]$, the yield of $(op)_2Cu^+$ was less than the initial concentration of $(op)_2Cu^{2+}$. This may be explained by a competition between $(op)_2Cu^{2+}$ and $(op)_2Cu^+$ for O_2^-:

$$(op)_2Cu^{2+} + O_2^- \rightarrow (op)_2Cu^+ + O_2 \tag{10}$$

$$(op)_2Cu^+ + O_2^- + 2H^+ \rightarrow (op)_2Cu^{2+} + H_2O_2 \tag{12}$$

Assuming steady state for the concentration of $(op)_2Cu^+$ and neglecting the back reactions −10 and −12 that are much slower, we obtain

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$$[(op)_2Cu^+]_{ss} = \frac{k_{10}[(op)_2Cu^{2+}]}{k_{12}} = \frac{k_{10}}{k_{10} + k_{12}}[(op)_2Cu^{2+}]_0 \quad (13)$$

d A plot of $OD_{435}/1$ vs. the initial concentration of $(op)_2Cu^{2+}$ is given in Figure 3. From the slope of the straight line we obtain

$$\frac{\epsilon_{435}k_{10}}{k_{10} + k_{12}} = 5.87 \times 10^3 M^{-1} cm^{-1} \quad (13a)$$

The determination of k_{12} from eq 13a is very inaccurate since $\epsilon = 7400 M^{-1} cm^{-1}$ is very close to the value of the slope; an error of 10% in ϵ can cause an error of 100% in k_{12}.

The above mechanism leads to the rate equation

$$-\frac{d[O_2^-]}{dt} = \frac{2k_{10}k_{12}}{k_{10} + k_{12}}[(op)_2Cu^{2+}]_0[O_2^-] \quad (14)$$

We define the "turnover" rate constant, $k_{cat.}$

$$k_{cat.} = \frac{2k_{10}k_{12}}{k_{10} + k_{12}} \quad (14a)$$

Since the absorption of $(op)_2Cu^{2+}$ in the UV region is too high, we were not able to follow the decay of O_2^- and to measure $k_{cat.}$ directly. Knowing the value of k_{10} and measuring k_{12} by other methods we can calculate ϵ_{435} and $k_{cat.}$ from eq 13a and 14a, respectively.

Reaction Mechanism for the Oxidation of $(op)_2Cu^+$ by O_2. We found that the rate law for the oxidation of $(op)_2Cu^+$ by molecular oxygen is first order with respect to $[O_2]$ and second order with respect to $[(op)_2Cu^+]$. Since in all solutions the concentration of O_2 is in excess relative to the $(op)_2Cu^+$ concentration, rate eq 15 is obtained. We found that k_{obsd} depends inversely on the

$$-d[(op)_2Cu^+]/dt = k_{obsd}[(op)_2Cu^+]^2 \quad (15)$$

concentration of $(op)_2Cu^{2+}$. When we added an excess of SOD relative to $(op)_2Cu^+$, the oxidation rate law for $(op)_2Cu^+$ was first order with respect to $[O_2]$ and $[(op)_2Cu^+]$. We found that boiled SOD has no influence on the oxidation rate law, and therefore we suggest, as Sigman did before,[12] that oxidation of $(op)_2Cu^+$ by O_2 proceeds via a superoxide intermediate.

We suggest the following reaction mechanism for the oxidation of $(op)_2Cu^+$ by O_2.

$$(op)_2Cu^+ + O_2 \underset{k_{10}}{\overset{k_{-10}}{\rightleftharpoons}} (op)_2Cu^{2+} + O_2^- \quad (10)$$

$$(op)_2Cu^+ + O_2^- + 2H^+ \xrightarrow{k_{12}} (op)_2Cu^{2+} + H_2O_2 \quad (12)$$

$$(op)_2Cu^+ + O_2 \underset{k_{-16}}{\overset{k_{16}}{\rightleftharpoons}} (op)_2CuO_2^+ \quad (16)$$

$$(op)_2CuO_2^+ + (op)_2Cu^+ + 2H^+ \xrightarrow{k_{17}} 2(op)_2Cu^{2+} + H_2O_2 \quad (17)$$

Assuming steady state for the concentration of O_2^- and $(op)_2CuO_2^+$, we obtain

$$-\frac{d[(op)_2Cu^+]}{dt} = \frac{2k_{-10}k_{12}[O_2][(op)_2Cu^+]^2}{k_{10}[(op)_2Cu^{2+}] + k_{12}[(op)_2Cu^+]} + \frac{k_{16}k_{17}[O_2][(op)_2Cu^+]^2}{k_{-16} + k_{17}[(op)_2Cu^+]} \quad (18)$$

Under the conditions where $k_{-16} > k_{17}[(op)_2Cu^+]$ and $k_{10} > k_{12}$, eq 18 reduces to eq 19 and second-order dependence on $(op)_2Cu^+$ concentration is observed. In Figure 4 there are two plots of

$$-\frac{d[(op)_2Cu^+]}{dt} = \left[\frac{2k_{-10}k_{12}[O_2]}{k_{10}[(op)_2Cu^{2+}]_0} + \frac{2k_{16}k_{17}[O_2]}{k_{-16}}\right][(op)_2Cu^+]^2 = k_{obsd}[(op)_2Cu^+]^2 \quad (19)$$

k_{obsd}/ϵ vs. $1/[(op)_2Cu^{2+}]_0$ in solutions, one saturated with air and the other with oxygen. The slopes and the intercepts of these straight lines are proportional to the concentration of oxygen in the solution. From the intercepts of the lines we obtained $k_{16}\cdot k_{17}/k_{-16} = (2.9 \pm 0.3) \times 10^8 M^{-1} s^{-1}$. From the slopes of the lines we obtained

$$k_{-10}k_{12}/k_{10} = 7 \times 10^3 M^{-1} s^{-1} \quad (19a)$$

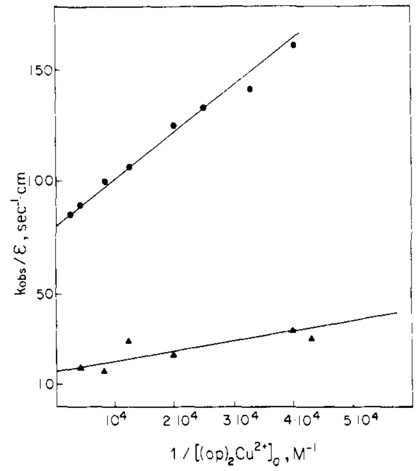

Figure 4. The dependence of the decay rate constant of $(op)_2Cu^+$ as a function of $1/[(op)_2Cu^{2+}]_0$. (●) Oxygen-saturated 0.05 M sodium formate at pH 7; (▲) air-saturated 0.05 M sodium formate at pH 7.

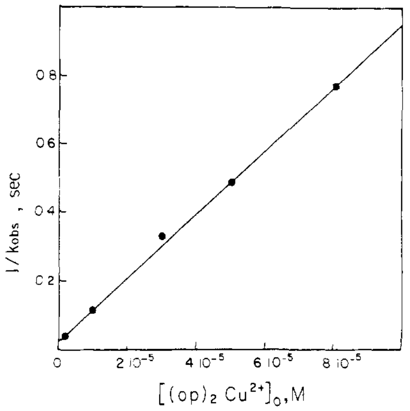

Figure 5. The reciprocal of the decay rate constant of $(op)_2Cu^+$ as a function of the initial concentration of $(op)_2Cu^{2+}$. All solutions contained 1.2 μM SOD and 0.05 M sodium formate at pH 7 and were oxygen saturated. The pulse duration was 0.1 μs.

When SOD is present it competes in a very efficient way with reactions 16 and 17. Using the same assumptions as for eq 18, we get

$$-\frac{d[(op)_2Cu^+]}{dt} = \frac{k_{-10}[O_2][(op)_2Cu^+](k_E\cdot[E]_0 + 2k_{12}[(op)_2Cu^+])}{k_{10}[(op)_2Cu^+]_0 + k_E\cdot[E]_0 + (k_{12} - k_{10})[(op)_2Cu^+]} + \frac{2k_{16}k_{17}[O_2][(op)_2Cu^+]^2}{k_{-16}} \quad (20)$$

k_E is the "turnover" rate constant of SOD, and as it is slightly different for SOD from different sources and batches, we measured it directly with our SOD and found $k_E = 3 \times 10^9 M^{-1} s^{-1}$. ($[E]_0$ is the initial concentration of SOD.) Under the conditions where the concentration of $(op)_2Cu^+$ is lower relative to the initial concentration of $(op)_2Cu^{2+}$ and SOD, eq 20 reduces to eq 21 and

$$-\frac{d[(op)_2Cu^+]}{dt} = \frac{k_{-10}k_E[E]_0[O_2][(op)_2Cu^+]}{k_{10}[(op)_2Cu^{2+}]_0 + k_E[E]_0} = k_{obsd}[(op)_2Cu^+] \quad (21)$$

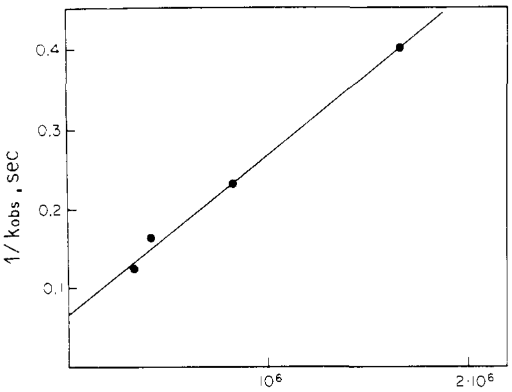

Figure 6. The reciprocal of the decay rate constant of $(op)_2Cu^+$ as a function of $1/[SOD]$. All solutions contained 20 μM $(op)_2Cu^{2+}$ and 0.05 M sodium formate at pH 7 and were oxygen saturated. Pulse duration was 0.1 μs.

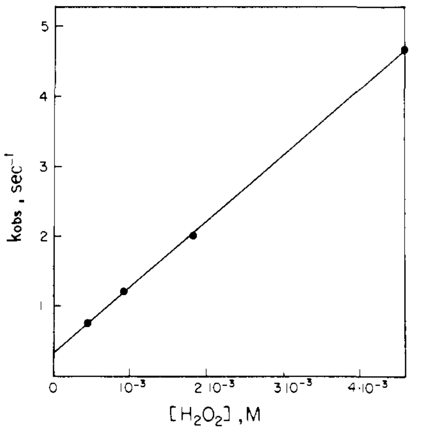

Figure 7. The rate constant of the decay of $(op)_2Cu^+$ as a function of $[H_2O_2]$. All solutions contained 30 μM $(op)_2Cu^{2+}$ and 0.05 M sodium formate at pH 7 and were air saturated. Pulse duration was 0.1 μs.

first-order dependence on $[(op)_2Cu^+]$ and on $[O_2]$ is observed. In Figure 5 the reciprocal of k_{obsd} is plotted vs. the initial concentration of $(op)_2Cu^{2+}$ at a constant concentration of SOD, and in Figure 6 the reciprocal of k_{obsd} is plotted vs. $1/[SOD]$ at a constant concentration of $(op)_2Cu^{2+}$. From the slopes of the two straight lines we derived the value of $K_{-10} = (5.0 \pm 0.3) \times 10^4$ M^{-1} s^{-1}.

Knowing the value of k_{-10}, we calculated from eq 19a $k_{12} = 2.7 \times 10^8$ M^{-1} s^{-1} and from eq 13a $\epsilon_{435} = 6990$ M^{-1} cm^{-1}. By reiteration we found the values $k_{12} = 2.95 \times 10^8$ M^{-1} s^{-1} and $\epsilon_{435} = 6770$ M^{-1} cm^{-1}. This value of ϵ_{435} is lower by 10% than that which was calculated from dosimetry.

Redox Potential of $(op)_2Cu^{2+}$. From the measured values of k_{10} and k_{-10} the equilibrium constant of reaction 10 was calculated to be $(3.86 \pm 0.3) \times 10^4$. Using the reduction potential $E^\circ_{O_2/O_2^-} = -0.33$ V,[23] we calculated the reduction potential $E^\circ_{(op)_2Cu^{2+}/(op)_2Cu^+} = -0.055$ V. With this value and the value of $E^\circ_{O_2^-/H_2O_2} = 0.89$ V,[23] we calculated $K_{12} = 5.63 \times 10^{15}$. Using the values of K_{12} and k_{12} we get $k_{-12} = 5.25 \times 10^{-8}$ M^{-1} s^{-1}.

The Oxidation of $(op)_2Cu^+$ by H_2O_2. When H_2O_2 is present in excess relative to $(op)_2Cu^+$ and O_2, the rate law for the oxidation of $(op)_2Cu^+$ was found to be first order with respect to $[(op)_2Cu^+]$ and $[H_2O_2]$.

$$(op)_2Cu^+ + H_2O_2 \rightarrow (op)_2Cu^{2+} + OH^- + OH\cdot \quad (22)$$

$$-\frac{d[(op)_2Cu^+]}{dt} = k_{22}[(op)_2Cu^+][H_2O_2] = k_{obsd}[(op)_2Cu^+] \quad (23)$$

Figure 7 gives the plot of k_{obsd} vs. the concentration of H_2O_2. From the slope of the straight line we measured $k_{22} = (937 \pm 20)$ M^{-1} s^{-1}.

$(op)_2Cu^{2+}$ as a Catalyst for Superoxide Dismutation. The mechanism of the dismutation of O_2^- catalyzed by copper phenanthroline is the same as that of SOD. The mechanism is given by eq 10 and 12. Using the values of k_{10} and k_{12} we calculate $k_{cat.} = (5.1 \pm 0.9) \times 10^8$ M^{-1} s^{-1}.

Most metal ions and complexes are not oxidized by O_2^- in aqueous solutions at rates competitive with that of superoxide. In other words, k_{12} for these substances is very small and $k_{cat.}$ calculated from eq 14a will be low. The probable explanation for this is that O_2^- is reduced only via a bridged, inner-sphere mechanism.[24] The $(op)_2Cu^{2+}$ complex has a heterocyclic nitrogen ligand similar to the histidine imidazole sites present in SOD.[25] The Cu(I) complex is not fully coordinated and therefore it can bind O_2^-. Hence, it is not surprising that copper phenanthroline can catalyze superoxide dismutation.

The catalytic properties of $(op)_2Cu^{2+}$ on superoxide dismutation have been investigated by other indirect methods. Superoxide was generated either from xanthine xanthine oxidase or from a solution of KO_2 with crown ether in Me_2SO.[26] The O_2^- reduces NBT^{2+} (nitroblue tetrazolium) to give the formazan. The formazan formation is followed spectrophotometrically at 540 nm. Substances with SOD activity compete for O_2^- and thus change the absorbance of the formazan. The SOD activity is determined by comparing the concentrations of these substances to the concentration of SOD, yielding 50% inhibition in the reduction of NBT^{2+}.[27,28]

Kimura et al.[26] found that the catalytic activity of $(op)_2Cu^{2+}$ on O_2^- dismutation is 2–3 orders of magnitude below our value. They determined $k_{cat.}$, using two different sources of O_2^- and the values obtained by their two methods differed by a factor of 9.[26] They also measured, for several other complexes of nickel and copper, $k_{cat.}$, where O_2^- was generated by the two methods. In those cases the two methods gave discrepancies of values, which differed by factors of 0.3–12.[26]

The SOD activity of the copper salicylate complex was determined by Kimura et al.[26] and by Younes et al.,[29] using the xanthine–xanthine oxidase system. The value obtained by Younes et al.[29] is 25-fold higher than that of Kimura et al.[26] Younes et al.[29] found that the SOD activity of copper salicylate determined by pulse radiolysis is 200-fold higher than the activity obtained by using the xanthine–xanthine oxidase method.

When indirect methods are used it is very difficult to discriminate between dismutation catalysis and the stoichiometric reaction of O_2^-. Moreover, the system is not well defined and some side reactions may occur. In the xanthine–xanthine oxidase system, the presence of hydrogen peroxide and inherent small amounts of metallic impurities can easily lead to Fenton-type reactions, and hence to misinterpretations of the observed results. It has been found[30] that the potassium superoxide system is not satisfactory for assaying SOD activity because of the interference by the absorption of O_2, dimisil ion ($CH_3SOCH_2^-$), which also mimics some of the chemistry of O_2^-, and K^+-crown ether complex, all of which appear to be present in variable amounts in solutions of KO_2.[30]

There are more examples where the SOD activity of various compounds was determined both by pulse radiolysis directly and

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by indirect methods. In all of these cases there were serious differences in the values of $k_{cat.}$ of up to several orders of magnitude.[29,31,32]

Therefore we believe that these indirect methods are not reliable, and direct methods of observing superoxide dismutase activity are to be preferred whenever possible.

Conclusion

We have demonstrated that $(op)_2Cu^{2+}$ is a good catalyst for superoxide dismutation with a "turnover" rate constant of $k_{cat.} = (5.1 \pm 0.9) \times 10^8$ M^{-1} s^{-1}, a value that is only six times lower than that of SOD.

We have determined, for the first time, the reaction mechanism of the oxidation of $(op)_2Cu^+$ by molecular oxygen in aqueous solution, a mechanism that proceeds via a superoxide intermediate. The redox potential of the $(op)_2Cu^{2+}$ complex was determined to be $E°_{(op)_2Cu^{2+}/(op)_2Cu^+} = -0.055$ V.

The biological damage of DNA in the presence of $(op)_2Cu^{2+}$ has been interpreted to be due to OH, being formed in reaction 22. In some of the studies published, there was not sufficient excess of H_2O_2 over O_2 to assure that most of the $(op)_2Cu^+$ will react with H_2O_2 and yield OH.[11,12,14] Therefore we conclude that $(op)_2Cu^+$ is possibly bound to DNA, and that the rate constants of relevant reactions of free and bound $(op)_2Cu^+$ may be different.

The binding of metal ions or their complexes to biological targets may change the chemical properties of the metal ion. The bound metal ion may play a large role, in both toxic[33] and pharmacological mechanisms.[33] The metal binding may also serve as a site-specific or site-directing mechanism.[34,35]

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Registry No. $(OP)_2Cu^+$, 17378-82-4; $(OP)_2Cu^{2+}$, 15823-71-9; O_2^-, 11062-77-4; H_2O_2, 7722-84-1.

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Effects of Secondary Bonding Interactions on the $[Fe_4S_4]^{2+}$ Core of Ferredoxin Site Analogues: $[Fe_4S_4(SC_6H_4\text{-}o\text{-}OH)_4]^{2-}$, a Distorted Cubane-Type Cluster with One Five-Coordinate Iron Atom

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Abstract: The series $[Fe_4S_4(SC_6H_4\text{-}o\text{-}X)_4]^{2-}$ with X = NH_2 (**1**), OMe (**2**), OH (**3**), and SMe (**4**) was prepared in order to examine the possible formation of five-coordinate Fe sites in cubane-type clusters with the biologically significant $[Fe_4S_4]^{2+}$ core oxidation level. The compound $(Et_4N)_2$(**3**) crystallizes in monoclinic space group $P2_1/c$ with a = 11.786 (3) Å, b = 20.701 (6) Å, c = 20.536 (7) Å, β = 94.42 (2)° and Z = 4. The cluster contains three conventional tetrahedral FeS_4 sites and one distorted trigonal-bipyramidal FeS_4O site, in which the $o\text{-}HOC_6H_4S^-$ ligand forms a chelate ring with the bond distances Fe–S = 2.313 (3) Å and Fe–O(H) = 2.318 (5) Å. The former bond, being only 0.035 Å longer than the mean of three other terminal Fe–S distances, represents a primary interaction; the Fe–OH bond distance reflects a weaker, secondary interaction. In the solid state the ^{57}Fe Mössbauer spectrum of **1** is a single quadrupole doublet, indicative of no bonding interactions with the NH_2 group. The spectrum of **3** consists of three doublets with a 1:2:1 intensity ratio, one of which has an unusually large isomer shift (δ = 0.51 mm/s at 4.2 K relative to Fe metal at this temperature). The spectrum of **4** was fit with two doublets in a 1:1 ratio; one of these has δ = 0.54 mm/s. On the basis of the structure of **3** the high velocity doublets are assigned to five-coordinate Fe atoms; parameters of the remaining doublets are usual for conventional $[Fe_4S_4(SR)_4]^{2-}$ clusters with tetrahedral FeS_4 sites. The spectrum of **2** is suggestive of very weak secondary interactions. Absorption spectral data and redox potentials are reported for **1**–**4** and para-substituted analogues of **1**–**3** in DMF solutions. On the basis of property comparisons with $[Fe_4S_4(SR)_4]^{2-}$ (R = Ph, $p\text{-}C_6H_4OH$), the high-energy charge-transfer band (440 nm) and anodically shifted potentials of cluster **3** are considered to result from O–H··S intraligand hydrogen bonding. The Mössbauer spectrum of **3** reveals that Fe–OH interactions are largely absent in frozen acetonitrile solution. Hence it is probable that secondary interactions are imposed properties of the crystalline state. The isomer shifts of doublets in the spectra of **3**, **4**, and the recently reported $[Fe_4S_4(SPh)_2(S_2CNEt_2)_2]^{2-}$ (having two five-coordinate sites) are the largest known for clusters with the 2+ core and are very similar to those (0.52–0.57 mm/s) of the P clusters of nitrogenase. The structural feature of five-coordination and associated secondary interactions are appropriate inclusions in formulations, and approaches to synthetic representations, of P clusters. At present this feature is the only known means of placing δ values of $[Fe_4S_4]^{2+}$ clusters in the range of the spectroscopically unique P clusters.

The chemistry of the cubane-type clusters $[Fe_4S_4(SR)_4]^{2-,3-}$ has been extensively developed, and the role of these clusters as structural and electronic analogues of oxidized (**1**) and reduced (**2**) ferredoxin protein sites has been demonstrated.[2-7] Among the leading properties of these clusters are reversible electron-transfer reactions, delocalized electronic structures with anti-

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