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Interaction between Copper(II)-Arginine Complexes and HO_2/O_2^- Radicals, a Pulse Radiolysis Study

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Abstract: Superoxide radicals were shown to react with all four Cu(II)-arginine complexes that exist between pH 1.5 and 12.5; $k_{10}(O_2^- + CuArgH^{2+}) = 3.3 \times 10^9\ M^{-1}\ s^{-1}$, $k_{12}(O_2^- + Cu(ArgH)_2^{2+}) = 1.3 \times 10^7\ M^{-1}\ s^{-1}$, $k_{13}(O_2^- + CuArg_2H^+) = 1 \times 10^6\ M^{-1}\ s^{-1}$, $k_{14}(O_2^- + CuArg^+) = 1 \times 10^8\ M^{-1}\ s^{-1}$. The system was studied in detail at pH 2.0 and pH ≥7. At low pH the mechanism was shown to proceed via a CuO_2H^{2+} complex while at higher pH a superoxide complex of the Cu(II)-arginine complexes was formed. The results are discussed in terms of the currently accepted mechanism by which SOD-Cu^{2+} dismutates O_2^- radicals.

Since the discovery that Cu,Zn superoxide dismutase (SOD-Cu^{2+}) catalyzes the dismutation of superoxide (O_2^-) radicals to oxygen and hydrogen peroxide,[1] the reactivity of Cu^{2+} complexes with O_2^- radicals has received much attention.[2-4] The general mechanism by which O_2^- reacts with Cu^{2+} ions has been postulated to involve the following equilibria and reactions:

$$Cu^{2+} + O_2^- \rightleftharpoons CuO_2^+ \rightleftharpoons Cu^+ + O_2 \qquad (I)$$

$$Cu^+ + O_2^- + 2H^+ \rightarrow Cu^{2+} + H_2O_2 \qquad (1)$$

or

$$CuO_2^+ + O_2^- + 2H^+ \rightarrow Cu^{2+} + H_2O_2 + O_2 \qquad (2)$$

Until recently, direct evidence for the formation of a CuO_2^+ complex in aqueous solution has not been demonstrated.[5] The extent to which a CuO_2^+ complex is stabilized likely depends upon the nature of the ligands that comprise a particular complex. An effect such as this has been demonstrated in studies of the reactions between Mn^{2+} and O_2^-, where the rate of disappearance of MnO_2^+ was shown to increase for the sequence of ligands: sulfate, formate, phosphate, pyrophosphate.[6] A recent theoretical study of the mechanism by which SOD-Cu^{2+} catalyzes the dismutation of O_2^- led to the postulation of an enzyme-O_2^- adduct that apparently is stabilized by a nearby arginine moiety of the enzyme.[7] This, along with a previous study of Cu(II)-histidine complexes,[4] motivated the present investigation of the interaction between O_2^- and Cu^{2+}-arginine complexes.

The structure of arginine ($H_2NC(=NH)NH(CH_2)_3(NH_2)COOH$) is such that it can function as either a bidentate or a tridentate ligand. An early study suggested that, in aqueous solution, arginine functions as a bidentate ligand, chelating through the carboxylic group and the α-amino group.[8] A recent crystallographic study verified this view and also showed that the copper-arginine complex is square planar with two arginines per copper.[9] In aqueous solution, this sort of bonding would leave the terminal amino group of the molecule free to act in a fashion similar to that postulated for SOD-Cu^{2+} and possibly stabilize a CuO_2^+ intermediate.

As indicated in equilibrium 1, high O_2 concentration would tend to promote formation of the CuO_2^+ intermediate. Therefore, the Cu(II)-arginine system was studied as a function of O_2 concentration as well as Cu(II) concentration.

Experimental Section

All solutions were prepared with water that had either been passed through a Millipore ultrapurification system or that had been triply distilled; no difference was observed between the two. Arginine (Sigma Chemical Co.), sodium formate, and copper sulfate (both from Merck Chemical Co.) were used without further purification.

Pulse radiolysis (pr) experiments were primarily carried out with the 10 MeV HRC linear accelerator at Risø National Laboratory which had been described previously.[10] Dosimetry was carried out with hexacyanoferrate(II) dosimeter with $G = 5.9$ and $\epsilon_{420nm} = 1000\ M^{-1}\ cm^{-1}$. The observed absorbance of O_2^- was then standardized to the dose with $\epsilon_{245nm} = 2300\ M^{-1}\ cm^{-1}$ at pH 7.[11] Additional experiments were carried out with the BNL 2 MeV Van de Graaff accelerator.[12] At BNL the $(SCN)_2^-$ dosimeter was used, assuming $G = 6.13$ and $\epsilon_{472nm} = 7950\ M^{-1}\ cm^{-1}$.

Experiments in the presence of high O_2 concentrations were carried out at Risø National Laboratory (Denmark), where the specially designed high-pressure cell allows the preparation of oxygenated solutions of up to 0.16 M. (This requires that the solution be put under 140 atm of O_2.)[13]

Results and Discussion

Water, when radiolyzed, yields[14]

$$H_2O \rightsquigarrow OH(2.75),\ e_{aq}^-\ (2.65),\ H\ (0.65),\ H_2O_2\ (0.70),\ H_2\ (0.45) \qquad (II)$$

where the values in parentheses are *G* values, that is the number of radicals produced per 100 eV of energy dissipated in water. Upon radiolysis of an O_2-saturated aqueous solution containing sufficient formate, all primary radicals are converted to HO_2/O_2^- radicals via a well-established mechanism.[15]

HO_2/O_2^- radicals disappear by a bimolecular process according to the following pH-dependent mechanism

$$HO_2 + HO_2 \rightarrow O_2 + H_2O_2 \qquad k_3 = 8.6 \times 10^5\ M^{-1}\ s^{-1} \qquad (3)$$

$$HO_2 + O_2^- + H_2O \rightarrow O_2 + H_2O + OH^- \qquad k_4 = 1 \times 10^8\ M^{-1}\ s^{-1} \qquad (4)$$

where the equilibrium between HO_2 and O_2^- radicals is defined as[11]

$$HO_2 \rightleftharpoons O_2^- + H^+ \qquad pK_5 = 4.8 \qquad (5,-5)$$

The reaction between HO_2/O_2^- radicals and Cu^{2+}-arginine complexes was studied as a function of pH under conditions such that the initial reaction was fast or assumed to be fast compared

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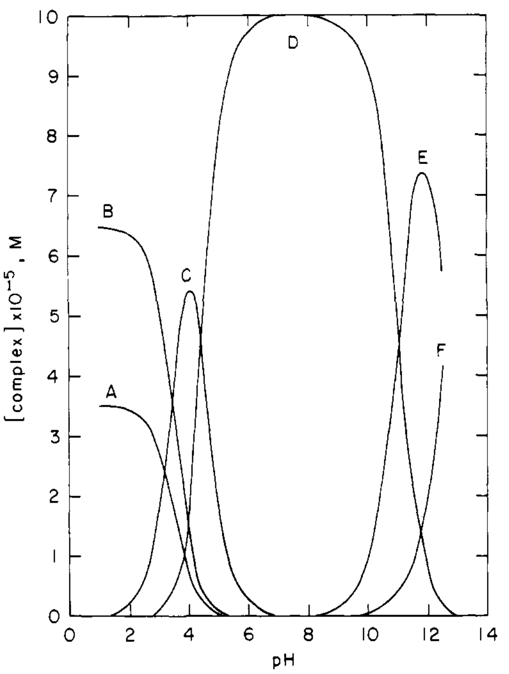

Figure 1. Equilibrium distribution of copper complexes as a function of pH, determined at 0.05 M formate, 0.01 M arginine, and 100 μM $CuSO_4$: A = $[Cu_{aq}^{2+}]$, B = $[Cu(HCO_2)^+]$, C = $[CuArgH^{2+}]$, D = $[Cu(ArgH)_2^{2+}]$, E = $[CuArg_2H^+]$, F = $[CuArg^+]$. All other copper complexes were present in less than 10^{-10} M concentrations.

Table I. Cumulative Stability Constants for Copper Complexes Formed in the Presence of Formate and Arginine

copper complex	log *K*	ref	copper complex	log *K*	ref
$CuArgH^{2+}$	19.40	8	$Cu(HCO_2)_3^-$	−0.15	3
$Cu(ArgH)^{2+}$	37.54	8	$Cu(HCO_2)_4^{2-}$	0.40	3
$CuArg_2H^+$	26.50	8	ArgH	11.50	16
$CuArg^+$	11.90	8	$ArgH_2^+$	20.55	16
$Cu(HCO_2)^+$	1.57	3	$ArgH_3^{2+}$	22.60	16
$Cu(HCO_2)_2$	0.65	3			

to all subsequent steps. The system was not studied with use of catalytic amounts of copper because, in some pH ranges, the copper complexes could not compete effectively with the spontaneous dismutation rate of HO_2/O_2^-. As the equilibrium distribution of Cu^+ complexes with pH is not known and as some of these complexes may react at extremely fast rates with HO_2/O_2^-, it was not possible to eliminate the occurrence of reactions 1 or 2 in all cases.

The equilibrium distribution of Cu^{2+} complexes of formate and arginine is illustrated in Figure 1. As is apparent, in the pH range of 1.5–12.5, six copper complexes are present in significant concentrations. All other Cu^{2+} complexes listed in Table I are present in concentrations less than 10^{-10} M and, therefore, do not perturb the kinetic and spectral characteristics of the system. The equilibrium distribution shown in Figure 1 was calculated by using known stability constants for these complexes[16,17] and a previously published computer program called COMICS,[18] assuming 0.05 M formate, 0.01 M arginine, and 0.0001 M $CuSO_4$. These concentrations will, henceforth, be considered as standard conditions, and any deviations from these concentrations will be noted.

As is apparent from Figure 1, there are six copper complexes that can react with HO_2/O_2^- radicals. Assuming that both HO_2 and O_2^- react with only two of the copper complexes, Cu_{aq}^{2+} and $Cu(HCO_2^+)$, the kinetic equation that describes the system involves eight rate constants and the concentrations of eight species at each pH studied. This is clearly not amenable to a solution in which a theoretical curve is fitted to the experimentally obtained rates of reaction as a function of pH. However, it is possible to vary the equilibrium distribution of the copper complexes at a specific pH in such a fashion that individual rate constants can be determined.

As the ligand (formate/arginine) is present in much higher concentration than the copper, changing the ligand concentration while maintaining constant copper concentration varies the ratios of the various copper complexes in equilibrium significantly. Therefore, in these studies, the ligand concentration was varied from 0.1 M formate/0.02 M arginine to 0.001 M formate/0.0002 M arginine while the copper concentration was constant at 0.1 mM. The ratio of formate to arginine was always held constant at 5-to-1 in order to ensure complete scavenging of the OH radical by formate.

At pH 2.0, the predominant species in equilibrium are Cu_{aq}^{2+} and $Cu(HCO_2)^+$. As the ligand concentration is varied the ratios of the two complexes vary significantly. However, the observed rate of reaction was proportional to the total concentration of both complexes, $k_{obsd} = 1.2 \times 10^8\ M^{-1}\ s^{-1}$. At this pH the ratio of $[HO_2]$ to $[O_2^-]$ is 625, suggesting that the reaction proceeds (because of the high rate) almost entirely via the HO_2 radical, yielding $k_6(HO_2 + Cu^{2+}) = k_7(HO_2 + Cu(HCO_2)^+) = 1.2 \times 10^8\ M^{-1}\ s^{-1}$.

At pH 4.5, the predominant species in equilibrium are Cu_{aq}^{2+}, $Cu(HCO_2)^+$, and $CuArgH^{2+}$. Assuming that, in analogy to the results obtained at pH 2.0, both Cu_{aq}^{2+} and $Cu(HCO_2)^+$ react with O_2^- at approximately the same rates, the system can be simplified and described by the following kinetic equation

$$k_{obsd} = \{k_6([Cu_{aq}^{2+}] + [Cu(HCO_2)^+])(H^+/K_5) + k_8([Cu_{aq}^{2+}] + [Cu(HCO_2)^+]) + k_{10}[CuArgH^{2+}]\}/\{1 + (H^+/K_5)\} \quad (III)$$

The use of $pK_5 = 4.8$, pH 4.5, and the previously determined rate, $k_6 = 1.2 \times 10^8\ M^{-1}\ s^{-1}$ leads, upon rearrangement, to

$$\underset{A}{\frac{3k_{obsd} - 2k_6([Cu_{aq}^{2+}] + [Cu(HCO_2)^+])}{[CuArgH^{2+}]}} = k_8 \underset{B}{\left[\frac{([Cu_{aq}^{2+}] + [Cu(HCO_2)^+])}{[CuArgH^{2+}]}\right]} + k_{10} \quad (IV)$$

A plot of A vs. B (Figure 2) yields a straight line with a slope of k_8 and an intercept of k_{10}. Experimental results lead to $k_8(O_2^- + Cu_{aq}^{2+}) = k_9(O_2^- + Cu(HCO_2)^+) = 1.14 \times 10^{10}\ M^{-1}\ s^{-1}$ and $k_{10}(O_2^- + CuHArg^{2+}) = 3.2 \times 10^9\ M^{-1}\ s^{-1}$. As the ratio of $[Cu_{aq}^{2+}]$ to $[Cu(HCO_2)^+]$ varies with the ligand concentration, the straight line shown in Figure 2 demonstrates that the previous assumption that k_8 is very close to k_9 is indeed valid. The values obtained here for k_8 and k_9 differ somewhat with values obtained previously,[2,3,19] deviations not considered significant in light of the complexity of this system and the differing ionic strengths at which they were measured.

At pH 6.0, the concentration of $CuArgH^{2+}$ varies relative to the ligand concentration while all of the other copper complexes do not change concentration significantly or are present in such low concentrations as to leave the system unperturbed. A plot of k_{obsd} vs. $[CuHArg^{2+}]$ yields a straight line with a slope equal to $2k_{10}$

$$CuHArg^{2+} + O_2^- \rightarrow \text{intermediate} \quad (10)$$

$$\text{intermediate} + O_2^- \rightarrow CuArgH^{2+} + O_2 + H_2O_2 \quad (11)$$

In this system, a reaction betwen cuprous ion or a CuO_2^+ complex and O_2^- is much faster than the reaction between cupric ion and O_2^- and, therefore, even at relatively high concentrations of copper

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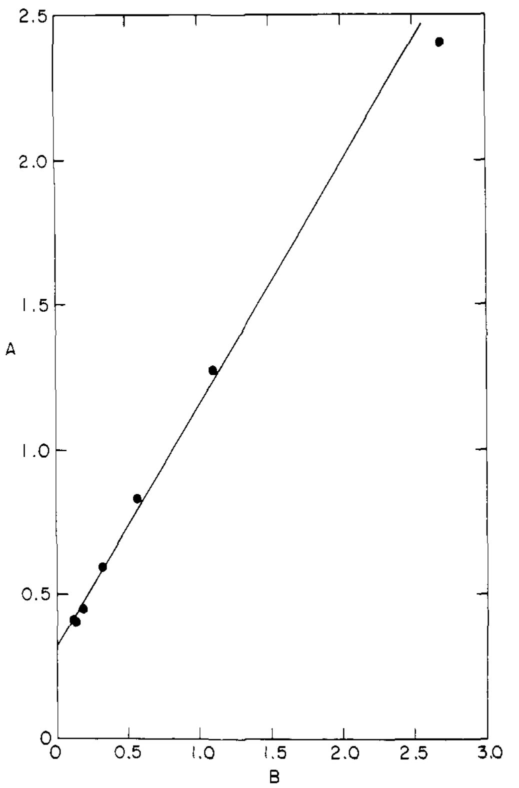

Figure 2. Determination of k_9 and k_{11} with eq IV. The slope yields $k_8 = k_9 = 1.4 \times 10^{10}$ M^{-1} s^{-1}. The intercept yields $k_{10} = 3.2 \times 10^9$ M^{-1} s^{-1}. Conditions: 100 μM Cu(II), O_2 saturated, variable formation and arginine (as discussed in the text).

(100 μM Cu^{2+}), steady-state conditions prevail and $2k_{10} = 6.6 \times 10^9$ M^{-1} s^{-1} is observed.

An overall rate equation was derived that describes the entire system as a function of pH and of the equilibrium distribution of the complexes at each pH.

$$k_{obsd}\ (s^{-1}) = \{k_6([Cu_{aq}^{2+}] + [Cu(HCO_2)^+])H^+/K_5 + k_8([Cu_{aq}^{2+}] + [Cu(HCO_2)^+]) + k_{10}[CuArgH^{2+}] + k_{12}[Cu(ArgH)_2^{2+}] + k_{13}[CuArg_2H^+] + k_{14}[CuArg^+]\}/\{1 + H^+/K_a\} \quad (V)$$

As described above, k_6, k_8, and k_{10} were already determined by studies at individual pHs. The remaining rate constants, k_{12}, k_{13}, and k_{14}, could only be obtained by fitting eq V to the observed rates of reaction as a function of pH (see Figure 3), yielding $k_{12}(O_2^- + Cu(ArgH)_2^{2+}) = 1.3 \times 10^7$ M^{-1} s^{-1}, $k_{13}(O_2^- + CuArg_2H^+) = 1 \times 10^6$ M^{-1} s^{-1}, and $k_{14}(O_2^- + CuArg^+) = 1 \times 10^8$ M^{-1} s^{-1}. As illustrated in Figure 3, the agreement between the observed rates as a function of pH and the theoretical line calculated from eq V is quite good.

Studies at pH 2. In a study of the dismutation of O_2^-/HO_2 by Cu_{aq}^{2+}, Rabani et al.[2] suggested that two distinct processes could be observed and that this indicated that copper might have formed a complex with the O_2^- prior to the electron-transfer step. Their conclusions, however, were based on very small absorbance changes and very short half-lives. Since, at low pH, the copper in our system is not present as an arginine complex (see Figure 1), we undertook a reinvestigation of this system as a function of both copper and oxygen concentrations.

At pH 2.0, although we do not observe the initial fast process described by Rabani et al.,[2] we observe subsequent processes similar to those reported therein (see Figure 4). Our initial process is first order in copper concentration and occurs at the rate discussed previously, $k_6 = k_7 = 1.2 \times 10^8$ M^{-1} s^{-1}. However, as seen in Figure 4, there is a process subsequent to the first process. The ratio of the observed absorbances corresponding to the two processed is roughly constant regardless of dose (O_2^- concentration), copper concentration, or oxygen concentration (see Figure 4, inset).

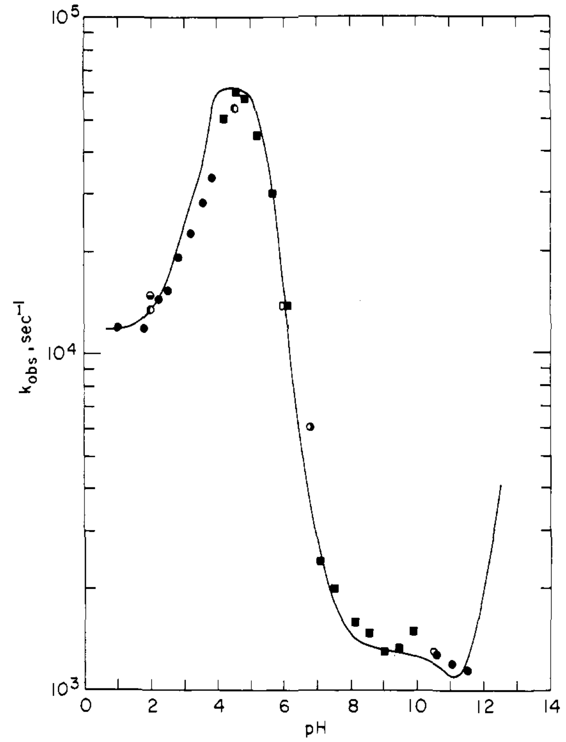

Figure 3. The symbols correspond to the observed rates of reaction as a function of pH. The different symbols correspond to data obtained at separate times. The solid line corresponds to the theoretical curve, calculated with eq V, as described in the text. The observed rates were measured in 0.05 M formate, 0.01 M arginine, and 100 μM $CuSO_4$.

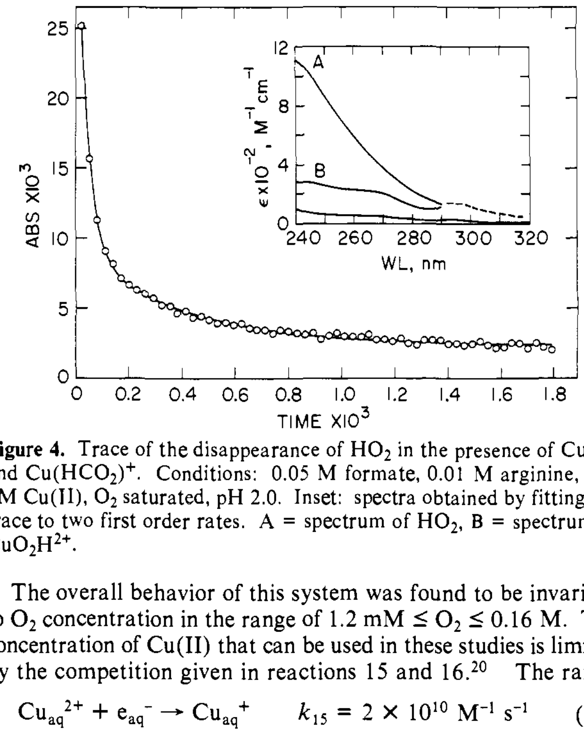

Figure 4. Trace of the disappearance of HO_2 in the presence of Cu_{aq}^{2+} and $Cu(HCO_2)^+$. Conditions: 0.05 M formate, 0.01 M arginine, 100 μM Cu(II), O_2 saturated, pH 2.0. Inset: spectra obtained by fitting the trace to two first order rates. A = spectrum of HO_2, B = spectrum of CuO_2H^{2+}.

The overall behavior of this system was found to be invariant to O_2 concentration in the range of 1.2 mM $\leq O_2 \leq$ 0.16 M. The concentration of Cu(II) that can be used in these studies is limited by the competition given in reactions 15 and 16.[20] The range

$$Cu_{aq}^{2+} + e_{aq}^- \rightarrow Cu_{aq}^+ \qquad k_{15} = 2 \times 10^{10}\ M^{-1}\ s^{-1} \qquad (15)$$

$$O_2 + e_{aq}^- \rightarrow O_2^- \qquad k_{16} = 2 \times 10^{10}\ M^{-1}\ s^{-1} \qquad (16)$$

of oxygen concentration given above meant that the system could be studied at 1 mM Cu(II) while still ensuring complete scavenging of the electron by O_2. Results of studies carried out at 25 μM $\leq$ Cu(II) $\leq$ 1 mM demonstrate that the behavior of this system is independent of [Cu(II)] in the range specified.

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The rate of disappearance of the transient, CuO_2H^{2+}, is $k_{17} = (2.25 \pm 0.34) \times 10^3\ s^{-1}$. A mechanism in which the Cu(II) reacts with HO_2 to form a CuO_2H^{2+} complex is in accordance with these results.

$$Cu_{aq}^{2+}/[Cu(HCO_2)]^+ + HO_2 \rightleftharpoons CuO_2H^{2+}/[Cu(HCO_2^-)(HO_2)]^+ \quad (6,-6)$$

$$[Cu(HCO_2^-)(HO_2)]^+/CuO_2H^{2+} \rightarrow Cu(I) + O_2 + H^+ \quad (17)$$

The transient cannot be Cu(I) as the reaction of Cu(I) with O_2 was shown previously[21] to occur at a rate of $10^6\ M^{-1}\ s^{-1}$ and it would thus disappear as soon as it was formed. If, in fact, Cu(I) disappears in this system at a slower rate than $10^6\ M^{-1}\ s^{-1}$, that rate should vary with $[O_2]$, and k_{18} was shown to be invariant to $[O_2]$. Also, the reaction between Cu(II) and CO_2^- was studied in both the absence and the presence of arginine as CO_2^- is known to reduce Cu(II) to Cu(I). The resultant spectra of Cu(I) varied with arginine concentration, indicating that while Cu(II) is not complexed by arginine at pH 2.0 Cu(I) does form such a complex. The spectrum attributed to Cu^+–arginine has a much higher molar absorbance than that shown in Figure 4, inset. If Cu^+–arginine is formed in small yield as a result of equilibrium processes, then the yield, and thus the spectrum, should change with variation of the species (O_2^-, O_2, Cu(II), arginine) in the system. As this is not the case, it is concluded that the transient is not Cu(I) but rather CuO_2H^{2+}. Another possibility is that the species is a dinuclear complex $[CuO_2HCu]^+$,[22] analogous to the species proposed in both manganous[6] and ferrous systems.[23] The independence of spectral and kinetic properties with $[Cu^{2+}]$ in the range described above makes this possible only if the dinuclear species is much more stable than the mononuclear species. A final possibility is that Cu(I) reacts with HO_2 to form CuO_2H^+. This can be excluded in that the system was shown to be invariant to $[Cu^{2+}]$. In the presence of a relatively large excess of Cu^{2+} (1 mM), HO_2 is consumed almost entirely in the first step, thus decreasing the probability of any HO_2 reacting with Cu^+. The transient, were it CuO_2H^+, would therefore be significantly reduced in concentration.

The formation of a CuO_2H^{2+} complex and/or a dinuclear derivative has been postulated previously.[24-30] In particular, evidence for its formation has been given, indirectly in pr studies[28] and in ESR studies.[29] In the ESR study, values of $K_6 = 5 \times 10^7\ M^{-1}$ and $k_{17} = 3 \times 10^1\ M^{-1}\ s^{-1}$ were published. The value of K_6 is, as they discussed, easily reconciled with our and Rabani's measured K_6 as, at these concentrations of Cu_{aq}^{2+}, the equilibrium is shifted completely to the right. The discrepancy between the values for k_{17} may arise from the experimental conditions as their experiments were carried out anaerobically and in the presence of H_2O_2 while the studies reported here were carried out at varying concentrations of O_2.

Studies at pH ≥7. The dismutation of O_2^- radicals by copper(II) arginine complexes in the alkaline pH range has been studied as a function of ligand and copper concentration, yielding the rate constants by which the different copper–arginine complexes react with superoxide. When this system was studied as a function of O_2 concentration at pH ≥7, however, a pronounced effect was observed on the spectral and kinetic properties of the system. As seen in Figure 5, increasing oxygen concentration leads to changes in absorbance as a function of time. At 1.2 mM O_2, the trace indicates that a single process occurs. At 160 mM O_2, two consecutive processes are observed. Studies with variable oxygen concentration demonstrate that the absorbance corresponding to the second process increases with increasing $[O_2]$. Furthermore, the ratio of the first absorbance to second absorbance appears independent of $[Cu^{2+}]$ and $[O_2^-]$ in the concentration range studied.

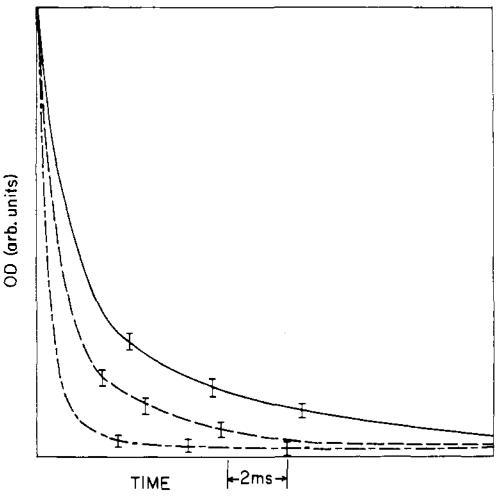

Figure 5. Traces of the disappearance of O_2^- in the presence of Cu(II)–arginine complexes. Conditions: 0.05 M formate, 0.01 M arginine, 100 μM Cu(II), pH 8.0, 270 nm. (---) 1.2 mM O_2 (O_2 saturated); (--) 60 mM O_2, (—) 85–160 mM O_2.

Since above pH 6 Cu(II)–arginine complexes absorb significantly in the low UV range, spectral studies were carried out between 270 and 290 nm, where O_2^- has relatively small molar absorbances ($\epsilon_{270nm} = 1450\ M^{-1}\ cm^{-1}$, $\epsilon_{290nm} = 520\ M^{-1}\ cm^{-1}$).[11]

The range of [Cu(II)] was limited as the initial reaction between Cu(II)–arginine and O_2^- is slow enough at pH 7–10 ($k \sim 10^6$–$10^8\ M^{-1}\ s^{-1}$) that the two aforementioned processes were not separable at [Cu(II)] < 100 μM. The competition between Cu(II)–arginine and O_2 for e_{aq}^- put an upper limit of 100–200 μM Cu(II) in O_2-saturated solution and 1–2 mM Cu(II) in a solution containing 160 mM O_2. At 100 μM Cu(II)–arginine and 160 mM O_2, both absorbances disappear by first-order processes, the first at approximately $10^3\ s^{-1}$ and the second at approximately $2 \times 10^2\ s^{-1}$, respectively. Both reactions appear to accelerate at higher Cu(II)–arginine concentrations.

These results can be explained by assuming the mechanism postulated in equilibrium I, that is the formation of a superoxide complex of Cu(II)–arginine as an intermediate. The equilibrium between CuO_2^+–arginine and ($Cu^+ + O_2$) explains the O_2 dependency of the formation of CuO_2^+–arginine. However, the overall mechanism must be more complex as the autoxidation of Cu(I) is not a simple process. In addition, the acceleration of both processes at high Cu(II)–arginine suggests that CuO_2^+–arginine may disappear by reacting with Cu^{2+}–arginine to form a binuclear complex, $[CuO_2Cu]^{3+}$.

Conclusions

This study is a continuation of an overall project that is aimed at the elucidation of the catalytic dismutation mechanism of superoxide radicals/perhydroxyl radicals by metals, metal complexes, and the active sites of superoxide dismutases. Numerous studies on the dismutation of HO_2/O_2^- by metal ions have shown that Cu^{2+} is the most efficient catalyst of the transition metals, whether in the aquo form, in some simple complexes, or as the central metal ion in a complex active site as in superoxide dismutases. The formation of corresponding short-lived transient complexes (CuO_2H^{2+}/CuO_2^+, $[CuHO_2Cu]^{4+}/[CuO_2Cu]^{3+}$, etc) in such systems have been, and still are, a central issue in the formulation of an overall reaction mechanism for the copper-catalyzed dismutation of superoxide radicals. Earlier investigations of such complexes as Cu(II)–histidine, Fe(II)–EDTA, etc., suggest that complexes which have at least one coordination site occupied by an exchangeable water molecule or OH^- ion react much faster

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with superoxide radicals than do fully chelated complexes. Accumulating evidence supports an earlier suggestion by Fee[31] that the rate-determining step in the overall mechanism may be the rate at which a liganded water/OH^- is exchanged for an O_2^-. For example, only one, ($CuHist_2H^{3+}$), out of six Cu(II)–histidine complexes known to exist in the pH range between 1 and 10 disproportionates superoxide radicals. This particular complex is known to have an exchangeable water molecule. Also, Fe(II)–EDTA has a coordinated OH^- ligand in the pH range 9–11.8 which can be exchanged with an O_2^- radical, this forming a Fe(II)–EDTA–O_2^- complex.[32,33]

The present study shows that although all four Cu(II)–arginine complexes that exist between pH 1.5 and 12.5 dismutate HO_2/O_2^-, the truly efficient ones are those two ($CuArgH^{2+}$ and $CuArg^+$) complexes that have only one arginine ligand, the other sites being occupied most likely with exchangable water or OH^- ions. The result of this selective reactivity, in both this and the Cu(II)–histidine system, is that these complexes carry out very facile reactions with superoxide over narrow pH ranges. This suggests that SOD may be biologically significant not only because it dismutates O_2^- at a diffusion-controlled rate but also because that rate is invariant of pH (pH 4.5–11).

The earlier study of the Cu(II)–histidine system led to the conclusion that the reactive complex, $CuHist_2H^{3+}$, did not form an observable transient with HO_2/O_2^-. In the present study, which is the first in which the reactivity of O_2^- with metal complexes was studied at high O_2 concentrations (0.16 M), again no HO_2/O_2^- complexes were observed when both Cu_{aq}^{2+} and Cu(II)–histidine were reacted with O_2^-. However, Cu(II)–arginine complexes were shown, in the presence of high O_2 concentration, to react through the formation of a CuO_2^+ intermediate. While these Cu(II)–arginine complexes should not be taken as model systems for superoxide dismutases, for which theoretical studies have advanced a hypothesis suggesting that the amino end of the arginine-141 group stabilizes an O_2^- bound to the copper in the active site (which is complexed by imidazole moieties), they show that O_2^- can be complexed by certain copper complexes, thus supporting the above hypothesis.

As postulated previously, the formation of a superoxide–copper complex in SOD may indeed be the answer to some complex structural and thermodynamic requirements for the efficient operation of the catalytic cycle.

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Registry No. $CuArgH^{2+}$, 108101-05-9; $Cu(ArgH)_2^{2+}$, 28471-34-3; $CuArg_2H^+$, 108101-06-0; $CuArg^+$, 97972-87-7; O_2^-, 11062-77-4.

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EXAFS and Near-Edge Structure in the Cobalt K-Edge Absorption Spectra of Metal Carbonyl Complexes

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Abstract: EXAFS data of the Co K-edges of $PPN[Co(CO)_4]$ (**1**), $Co_3(CO)_9CH$ (**2**), and $Co_4(CO)_{10}(PPh)_2$ (**3**) have been analyzed. A spherical wave theory using ab initio phase shifts and including multiple scattering to third order allows structural parameters to be accurately obtained. Distances were determined to within 0.03 Å with use of data from 14 eV above the absorption edge. This method can also provide an estimate of the mean Co–C–O angle, but an attempt to reproduce multiple scattering effects with a variable coordination number and phase parameter was unreliable in this regard. Intraligand multiple scattering is dominant in these complexes. Statistical criteria for limiting the number of refinable parameters in a multi-shell system are also presented. Pre- and near-edge features may be used qualitatively to fingerprint the cobalt environments in these complexes.

Previously we have used EXAFS to identify the structures of metal carbonyl clusters[2] and to study the chemistry of clusters on functionalized and nonfunctionalized oxide surfaces.[3-5] While these treatments employed a spherical wave formalism,[6] using a computationally fast algorithm suitable for polycrystalline or amorphous samples,[7] rather than the more commonly used plane wave approximation,[8] they were restricted to single scattering events. Although multiple scattering is most important at low

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