The Copper–Poly-L-histidine Complex. I. The Environmental Effect of the Polyelectrolyte on the Oxidase Activity of Copper Ions

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Abstract: The copper-catalyzed oxidation by molecular oxygen of ascorbate and homogentisate ions, hydroquinone as well as tetramethyl-*p*-phenylenediammonium and 1-(2,5-dihydroxyphenyl)isopropylammonium cations, was studied in the presence of polyhistidine (PLH). It was found that the addition of PLH enhances the catalytic efficiency of Cu(II) toward negatively charged and neutral substrates, but inhibits it toward positively charged substrates. The PLH–Cu(II) unlike the Cu(II)-catalyzed reaction becomes zero order in substrate concentration at relatively low concentration of substrate and exhibits Michaelis–Menten kinetics. The specific effect of PLH on the Cu(II)-catalyzed reactions, which is abolished in the presence of moderate concentration of inert electrolytes, is interpreted by the electrostatic effect of the polyelectrolyte on the concentration of the reactants involved in the close vicinity of the bound copper. The analogy between the PLH–Cu(II)-catalyzed reactions and copper-containing oxidases is discussed.

Copper ions have been shown to act as catalysts in different oxidations of organic substrates by molecular oxygen. It has been shown that the complexation of Cu(II) by certain ligands modifies its catalytic activity. Ligands such as azide, dipyridyl, or *o*-phenanthroline, which stabilize monovalent copper, have been shown to increase the catalytic activity of copper by a factor of 1.2–3.5 when present in an equivalent amount to the metal.[2] This effect may be attributed to a linear free energy relationship between the ΔF of the oxidation reaction

$$\text{Cu(II)} + \text{substrate (reduced)} \longrightarrow \text{Cu(I)} + \text{substrate (oxidized)}$$

and its energy of activation. This reaction is rate determining since the oxidation of Cu(I) to Cu(II) by molecular oxygen is a relatively very fast process, even when the Cu(I) is complexed by ligands which stabilize this valency.[3]

In certain oxidation reactions by molecular oxygen the Cu(II)–poly-L-histidine complex was found to exhibit a higher catalytic activity than copper ions in their aquo or acetato complexes.[4] As imidazole and histidine did not show an analogous behavior,[4] it seems improbable that the observed effect is due to a change in the oxidation–reduction potential of Cu(II). Thus a different interpretation of this specific catalytic effect was desired. It was the purpose of this study to investigate the modified catalytic activity of copper when bound to the polyamino acid. Since imidazole residues were suggested as ligands for the copper within the active site of copper-containing oxidases,[5] it was hoped that the understanding of the copper–polyhistidine system might throw some light on certain enzymic oxidase activities.

(1) The Soreq Research Establishment, Yavneh, Israel.
(2) V. S. Butt and M. Hallaway, *Arch. Biochem. Biophys.*, **92**, 94 (1961); S. Isaka, *Nature*, **179**, 578 (1957).
(3) M. Anbar and I. Pecht, to be published.
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Experimental Section

Materials. All solutions were prepared from distilled water which was redistilled from alkaline permanganate and then from phosphoric acid in all-glass apparatus. All reagents were copper free as determined by the biquinoline method.[6] Poly-L-histidine (PLH)[7] was a product of Yeda Research and Development Co., Rehovoth. The polymer was dried *in vacuo* at 100° over P_2O_5 to constant weight. The molecular weight determined by sedimentation velocity and diffusion was 10,000 ± 100.

Anal. Calcd for $(C_6H_7ON_3)_n \cdot 0.5nH_2O$: N, 28.8. Found: N, 28.7.

$CuSO_4 \cdot 5H_2O$ and ascorbic acid were BDH Analar reagents. Catechol and *p*-H_2Q[7] were Fluka puriss, p.a. reagents and were used without further purification. HGA[7] obtained from Fluka (purum grade) was recrystallized twice from dioxane–benzene (1:3) and dried *in vacuo* at 40° to constant weight, mp 152°. TMPD[7] dihydrochloride obtained from Fluka (purum grade) was recrystallized twice from water with ethanol in the cold. The material was washed with absolute ether and dried over H_2SO_4 at room temperature.

1-(2,5-Dihydroxyphenyl)isopropylamine was prepared from the corresponding dimethyl ether, kindly supplied by Polaroid Co.[8] according to the following procedure: 10 g of 1-(2,5-dimethoxyphenyl)isopropylamine was mixed with 100 g of 48% HBr. The mixture was heated and stirred under nitrogen until about 25 ml of the distillate was collected at maximum temperature of 125°. Reflux was then maintained for 24 hr, after which the volatiles were removed *in vacuo*. The residue was dissolved in 25 ml of water, adjusted to pH 3.0 with $NaHCO_3$, treated with charcoal, and filtered under nitrogen. This furnished a brownish solution of the free amine hydrobromide. Owing to susceptibility to atmospheric oxygen, it was difficult to isolate the compound in a pure state; thus appropriate dilutions of the purified stock solution were made up for the kinetic runs.

Methods. All spectrophotometric determinations were made on a Zeiss PMQ-II thermostated (25°) spectrophotometer equipped with a Honeywell, 10-mv recorder.

Assay Procedure. All oxidations of the substrates were conducted in quartz cuvettes in a final volume of 3.0 ml containing the components as specified below. The solutions were saturated with pure oxygen by bubbling prior to addition of the substrate.

(6) G. Felsenfeld, *Arch. Biochem. Biophys.*, **87**, 247 (1960).
(7) The following abbreviations are used: poly-L-histidine = PLH; *p*-hydrobenzoquinone = *p*-H_2Q; *p*-benzoquinone = Q; N,N,N′,N′-tetramethyl-*p*-phenylenediamine = TMPD; oxidized TMPD = TMOX; homogentisic acid = HGA; 1-(2,5-dihydroxyphenyl)isopropylamine = ipH_2Q.
(8) The authors wish to thank Dr. Sheldon A. Buckler, Manager of the Pilot Chemicals Department of Polaroid Corp. Research Laboratory, for the dimethoxy derivative and for the detailed procedure of preparing the dihydroxy compound.

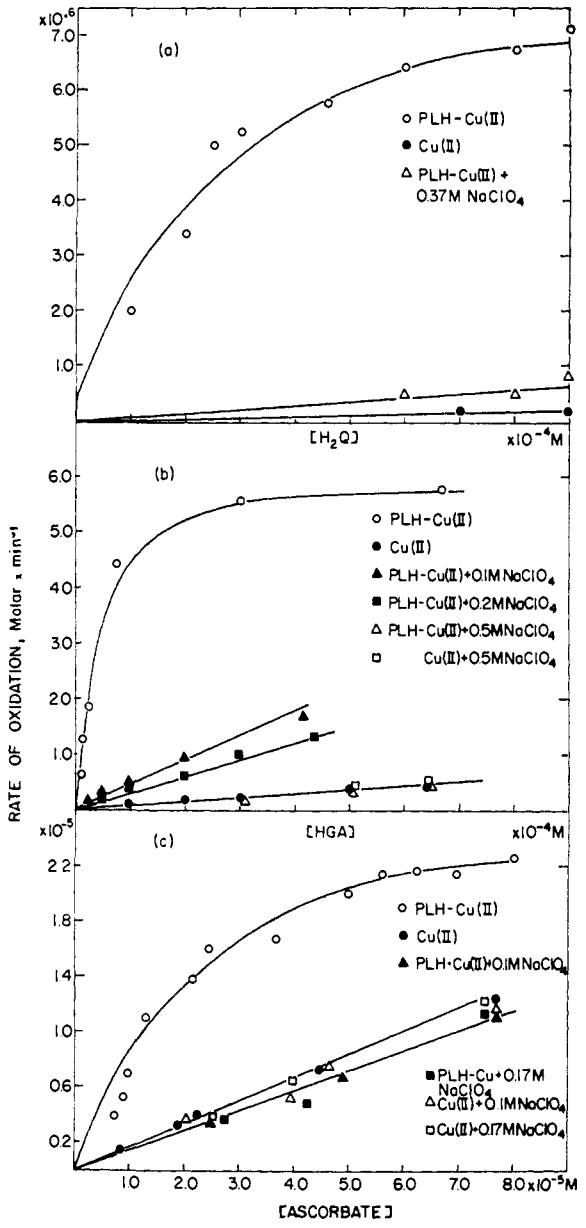

Figure 1. Rate of oxidation as a function of the substrate concentration: a, H_2Q oxidation in sodium acetate buffer, 0.02 *M*, pH 4.8, Cu(II) 8 × 10^{-6} *M*, PLH 4 μg/ml (4 × 10^{-7} *M* in polymer or 2.65 × 10^{-5} in histidyl residues); b, HGA oxidation in sodium acetate buffer, 0.02 *M*, pH 5.0, Cu(II) and PLH concentration same as in a; c, ascorbate oxidation in sodium acetate buffer, 0.02 *M*, pH 4.3, Cu(II) 6 × 10^{-6} *M*, PLH 6 μg/ml (4 × 10^{-5} *M* in histidyl residues).

In the kinetic experiments, initial rates were determined for each substrate concentration. All kinetics were followed at 25°.

a. Ascorbic Acid. The rate of ascorbate oxidation was followed spectrophotometrically at 265 mμ as described earlier.[9] The molar extinction coefficient of ascorbic acid was determined within the pH range 4.0–5.9 using 0.02 *M* sodium acetate buffer. The measured extinction coefficients (Table I) agree with those reported earlier for only a limited number of pH values.[10]

b. *p*-Hydroquinone. *p*-Hydroquinone oxidation to the quinone was followed spectrophotometrically at 240 mμ. H_2Q has a molar extinction coefficient of ε 475 M^{-1} cm^{-1} at this wavelength and the corresponding quinone has ε 2454 M^{-1} cm^{-1}.[11] These values do not vary significantly in the pH range 4.0–5.9. The change in *p*-H_2Q concentration was calculated according to the equation

$$\Delta[p\text{-}H_2Q] = \Delta A^{240}{}_{obsd}/(\epsilon^{240}{}_{Q} - \epsilon^{240}{}_{H_2Q})$$

where $\Delta A^{240}{}_{obsd}$ is the change in absorbance at 240 mμ, and $\epsilon^{240}{}_{Q}$ and $\epsilon^{240}{}_{H_2Q}$ are the molar extinction coefficient of the quinone and the hydroquinone, respectively.

c. Catechol. *o*-Benzoquinone formation was checked at 390 mμ.[12]

d. N,N,N′,N′-Tetramethyl-*p*-phenylenediamine. The oxidation of TMPD was followed at 257 mμ where ε is 14,260 M^{-1} cm^{-1}. The oxidized product ("purple product") has ε 5500 M^{-1} cm^{-1} at this wavelength. Extinction coefficients of both substances do not vary significantly in the pH range 4.0–5.9. The decrease in the TMPD concentration was calculated from

$$\Delta[TMPD] = \Delta A^{257}{}_{obsd}/(\epsilon^{257}{}_{TMPD} - \epsilon^{257}{}_{TMOX})$$

where $\Delta A^{257}{}_{obsd}$ is the observed change in absorbance at 257 mμ.

e. Homogentisic Acid. The oxidation of homogentisic acid was followed spectrophotometrically by observing the formation of 1,4-benzoquinone-2-acetic acid at 250 mμ. The extinction coefficient of this product was found to be ε 17,900 M^{-1} cm^{-1}, which is different from that reported earlier.[13] The extinction coefficient at 290 mμ for the homogentisic acid was identical with that reported earlier.[13]

f. 1-(2,5-Dihydroxyphenyl)isopropylamine. The oxidation of ipH_2Q was followed by the increase in absorbance at 288 mμ. Since the compound could not be isolated, its concentration was determined by micro-Kjeldahl nitrogen analysis of the stock solutions.[14]

Determination of H_2O_2. Hydrogen peroxide was determined by the triiodide[15] or the titanium peroxy complex[16] methods.

Table I. Molar Extinction Coefficient of Ascorbic Acid

pH	ε, M^{-1} cm^{-1} at 265 mμ
3.40	6,500
3.90	10,000
4.40	13,000
4.80	13,600
5.45	14,300
5.85	14,300

Results

Ascorbic Acid and Hydroquinone Oxidations. The rate of oxidation of ascorbic and homogentisic acids and of hydroquinone by PLH–Cu(II) was followed as a function of the substrate concentration (Figure 1). The results were compared with the catalytic effect of the same concentrations of Cu(II) in the absence of PLH. The rate of oxidation catalyzed by PLH–Cu(II) becomes independent of substrate concentration at relatively low concentrations of the substrates. This typical Michaelis–Menten kinetic behavior,[17] which indicates the existence of a catalyst–substrate complex, is not exhibited by the aquoacetato complex in the same range of substrate concentrations. Assuming the existence of a catalyst–substrate complex as an intermediate

$$\text{substrate} + \text{PLH–Cu(II)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} (\text{substrate–PLH–Cu(II)}) \xrightarrow{k_3} \text{products}$$

(9) E. Racker, *Biochim. Biophys. Acta*, **9**, 577 (1952).
(10) C. Dalgiesh, *Biochem. J.*, **49**, 635 (1951).
(11) J. H. Baxendale and H. R. Hardy, *Trans. Faraday Soc.*, **50**, 808 (1954).
(12) H. S. Mason, *J. Biol. Chem.*, **181**, 803 (1949).
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(15) A. O. Allen, C. J. Hochanadel, J. A. Ghormley, and T. W. Davies, *J. Phys. Chem.*, **56**, 575 (1952).
(16) A. Weissler, *Ind. Eng. Chem., Anal. Ed.*, **17**, 695 (1954).
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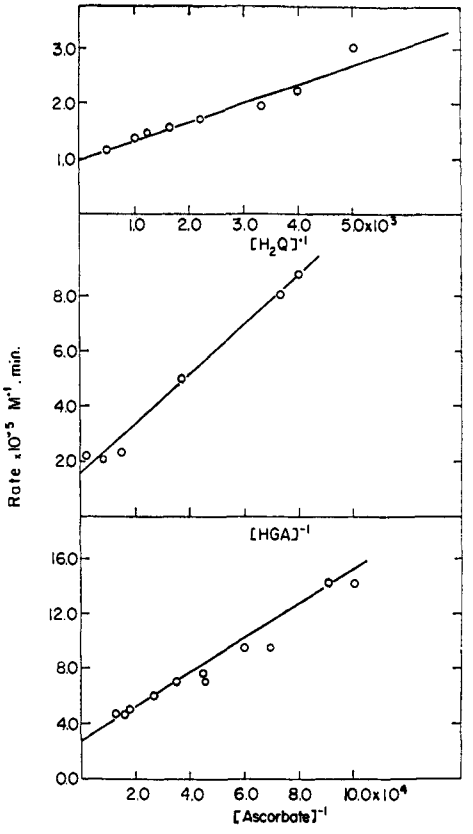

Figure 2. Lineweaver-Burk plot of the PLH-Cu(II)-catalyzed oxidations. Experimental details as under Figure 1.

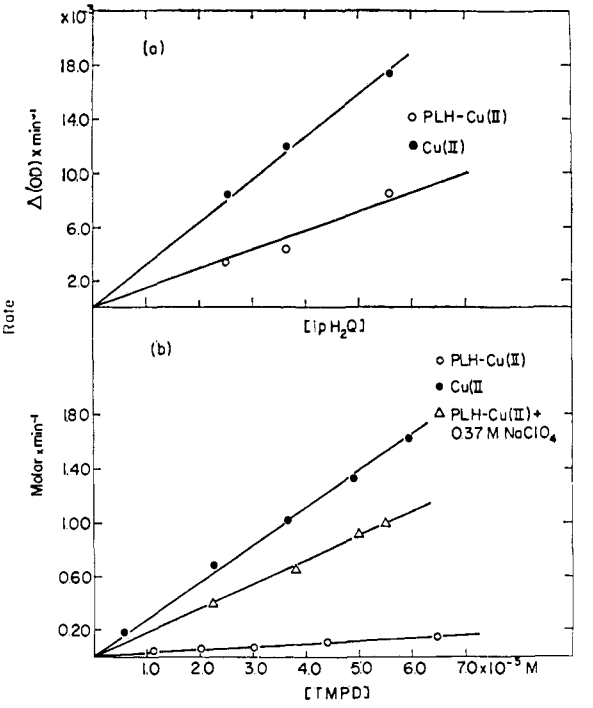

Figure 3. Rate of TMPD and ipH_2Q oxidation as a function of substrate concentration: a, ipH_2Q oxidation in sodium acetate buffer, 0.02 *M*, pH 5.0, Cu(II) 1.67×10^{-5} *M*, PLH 9.5 μg/ml (9.5×10^{-7} *M* in polymer or 6.4×10^{-5} *M* in histidyl residues); b, TMPD oxidation in sodium acetate buffer, 0.02 *M*, pH 5.0, Cu(II) 8×10^{-6} *M* and PLH 4 μg/ml.

(which obeys the steady-state approximation d[substrate–PLH–Cu(II)]/dt = 0), one obtains

$$\text{rate of oxidation} = R = \frac{k_3[\text{PLH–Cu(II)}][\text{substrate}]}{\frac{k_2 + k_3}{k_1} + [\text{substrate}]} \quad \text{(I)}$$

$$\frac{1}{R} = \frac{1}{k_3[\text{PLH–Cu(II)}]} + \frac{k_1 + k_3}{k_1 k_3[\text{PLH–Cu(II)}][\text{substrate}]} \quad \text{(II)}$$

The plot of the reciprocal of the rate as a function of the reciprocal of the substrate concentration should give, therefore, a straight line. From the intercept of this line and its slope one may derive k_3, the specific rate of decomposition of the substrate–catalyst complex, and the Michaelis constant, K_m[18] for this reaction. The rate of the PLH–Cu(II)-catalyzed reaction, when plotted according to the above cited Lineweaver–Burk treatment,[19] yields a straight line (Figure 2). Values of K_m, V_m,[18] and k_3 (k_3 is also known as "turnover number," moles of substrate transformed per mole of catalyst per minute) are summarized in Table II.

Under the experimental conditions catechol was not oxidized at an observable rate by either PLH–Cu(II) or Cu(II) within the pH range 4.0–5.8.

TMPD and ipH_2Q Oxidation. The oxidations of TMPD and ipH_2Q, which are positively charged in the pH range studied, were investigated (Figure 3). The results point to major differences between the positively and negatively charged substrates. First, Michaelis–Menten kinetics is not observed for the PLH–Cu(II) catalyzed oxidation of ipH_2Q and TMPD. Further, the rate of oxidation of TMPD and ipH_2Q is higher when catalyzed by "free" Cu(II) than by the PLH–Cu(II) complex. The kinetic parameters for these oxidations are also summarized in Table II.

Effect of pH. The effect of pH on the catalyzed oxidations is presented in Figure 4. Whereas the rate of Cu(II)-catalyzed oxidations increases with $1/[H^+]$ throughout the measured range, the PLH–Cu(II)-catalyzed reaction shows a different behavior. In this case the rate increases with pH to about pH 5 and then levels off, reaching a limiting value. In the case of ascorbate the rate of oxidation reaches another lower limiting value below pH 3.8, which is explained by the fact that ascorbic *acid* (pK = 4.2) does not undergo appreciable oxidation by copper ions.

Effect of Ionic Strength. From the results (Figure 1) one observes that polyhistidine increases the catalytic efficiency of Cu(II) in the case of negatively charged and neutral substrates, but inhibits the Cu(II)-catalyzed oxidation of positively charged substrates (Table II). Since imidazole and histidine do not affect the catalytic properties of copper it was suggested that the effect of the positively charged polyelectrolyte on the catalytic efficiency of copper is of *electrostatic* nature. In order to clarify this point, the effect of ionic strength on the rate of oxidation was investigated. The results are summarized in Figures 1 and 3. Sodium perchlorate was chosen since ClO_4^- ions do not form stable complexes with monovalent or bivalent copper.

Oxidations induced by the aquoacetato complex of copper were found to be unaffected by inert salts. The addition of moderate concentrations of inert salts modifies the kinetic behavior of the PLH–Cu(II)-

(18) $K_m = (k_2 + k_3)/k_1$; $V_m = k_3$[PLH–Cu(II)].
(19) H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, **56**, 658 (1934).

Table II. Kinetic Parameters for the PLH–Cu(II)-Catalyzed Oxidation

Substrate	pH of assay	K_m, M	V_{max}, $M \times min^{-1}$	k_3,[a] min^{-1}	k_{obsd},[b] min^{-1} PLH–Cu(II)	k_{obsd},[b] min^{-1} Cu(II)
Ascorbic acid	4.3	4.3×10^{-5}	3.6×10^{-5}	6.0	1.0	0.16
Homogentisic acid	5.0	6.0×10^{-5}	0.7×10^{-5}	0.88	0.1	0.00058
p-Hydroquinone	4.8	4.0×10^{-4}	1.1×10^{-5}	1.37	0.19	0.002
TMPD	4.9	...	...	...	0.025	0.25

[a] k_3 (turnover number) = moles of substrate/mole of copper × min. [b] Calculated graphically from the slope of the linear parts of Figure 1

catalyzed reactions which then approaches that of the Cu(II) acetate complex. The Michaelis–Menten behavior disappears, and the rate becomes equal to that of the Cu(II)-catalyzed reaction.

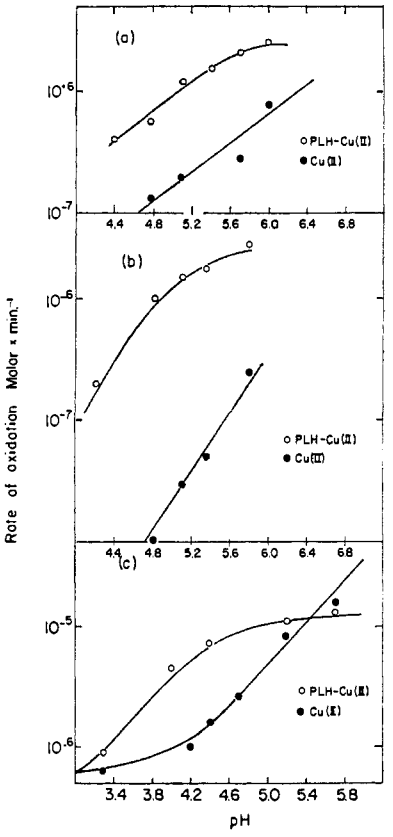

Figure 4. Dependence of oxidation rate on pH: a, H_2Q oxidation in 0.02 M sodium acetate buffer, Cu(II) 8×10^{-6} M, PLH 4 μg/ml, H_2Q 1×10^{-3} M; b, HGA oxidation in 0.02 M sodium acetate buffer, Cu(II) 8×10^{-6} M, PLH 4 μg/ml, HGA 2×10^{-5} M; c, ascorbic acid oxidation in 0.02 M sodium acetate buffer, Cu(II) 6×10^{-6} M, PLH 6 μg/ml, substrate concentration at 1.1×10^{-5} M.

In the case of the positively charged TMPD the rate of oxidation of the substrate by the PLH–Cu(II) complex *increases* at high ionic strength.

Products of Oxidation. In each of the oxidation reactions investigated, hydrogen peroxide could be found as a reduction product of oxygen. The hydrogen peroxide is formed in an amount equivalent to that of the substrate oxidized. Since H_2O_2 formed during the reaction can participate in side reactions, *initial* rates were always determined. In order to check on the possibility of extensive side reactions, H_2S or $NaBH_4$ was introduced into oxidized reaction mixtures, and the original substrates were assayed. Quantitative recovery of the reduced substrate was obtained in all cases.

Discussion

It has been demonstrated that binding of Cu(II) to polyhistidine enhances the catalytic activity of copper in the oxidation of neutral and of negatively but not positively charged substrates. In the latter case an inhibitory effect was observed on complexation with the positively charged polyelectrolyte. Since the PLH–Cu(II) complex, which is readily formed at pH 3.0–4.0, has a dissociation constant[20] of approximately 10^{-18}, practically all the copper ions are bound to the PLH. The kinetic behavior of the PLH–Cu(II)-catalyzed reactions points to the existence of an intermediate, PLH–Cu(II) substrate. The oxidation reaction, which is rate determining, takes place intramolecularly in this intermediate. The formation of such a substrate–PLH–Cu(II) complex with positively charged substrates is inhibited by electrostatic repulsion.

The mechanism of the copper-catalyzed reactions may be described by the following steps

$$Cu^{2+} + H_2A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} CuAH^+ + H^+ \quad (1)$$

$$CuAH^+ \xrightarrow{k_3} Cu^+ + AH \quad (2)$$

$$Cu^+ + O_2 \xrightarrow{\text{fast}} Cu^{2+} + O_2^- \quad (3)$$

where H_2A represents the substrate and AH the radical produced on single equivalent oxidation.

Reaction 2 is the rate-determining step whereas reaction 1 is a preequilibrium. Reaction 3 was shown to proceed at a rate much faster than the oxidations measured in this study.[3]

Hydrogen peroxide, which was found to be formed stoichiometrically, is most probably produced by reaction 4 which follows reaction 3.

$$Cu(I) + O_2^- + 2H^+ \longrightarrow Cu(II) + H_2O_2 \quad (4)$$

Under conditions where the intermediate complex $CuHA^+$ fulfills the steady-state approximation, the rate of reaction will be expressed by

$$R = -\frac{d[H_2A]}{dt} = \frac{k_1k_3[Cu^{2+}][H_2A]}{k_2[H^+] + k_1[H_2A] + k_3} \quad (III)$$

Under the experimental conditions $(k_2[H^+] + k_3) >> k_1[H_2A]$; thus for the Cu(II) aquoacetato catalyzed reaction at constant pH, $R \sim [H_2A]$ as shown in Figures 1 and 3. In all cases studied R increases with pH, but only in the case of homogentisate ions $R \sim 1/[H^+]$, which implies that under the experimental conditions $k_2[H^+] >> (k_3 + k_1[H_2A])$; thus the observed specific rate constant (k_{obsd}, Table II) is equal to $k_1k_3/k_2[H^+]$. For the other substrates $k_{obsd} = k_1k_3/(k_2[H^+] + k_3)$, most probably because k_3 has a larger value (Table II).

(20) A. Levitzki, H. A. Saroff, A. Berger, and M. Anbar, to be published.

In the case of the PLH–Cu(II)-catalyzed reaction, the analogous equations will be

$$\text{PLH–Cu(II)} + \text{H}_2\text{A} \underset{k_{2a}}{\overset{k_{1a}}{\rightleftharpoons}} \text{PLH–Cu(II)–AH} + \text{H}^+ \qquad (1a)$$

$$\text{PLH–Cu(II)–AH} \xrightarrow{k_{3a}} \text{PLH–Cu(I)} + \text{AH} \qquad (2a)$$

In this case

$$R = \frac{k_{3a}[\text{PLH–Cu(II)}][\text{H}_2\text{A}]}{[k_{2a}[\text{H}^+] + k_{3a}]/k_{1a} + [\text{H}_2\text{A}]} \qquad (\text{IV})$$

Formula IV is presented in a form similar to formula I, which was shown to describe the experimental behavior of the PLH–Cu(II)-catalyzed reaction.

It should be noted that $k_{2a}[\text{H}^+] = k_2$ of formula I. The PLH–Cu(II)-catalyzed reaction follows Michaelis–Menten kinetics because the terms $k_{2a}[\text{H}^+] + k_{3a}$ and $k_{1a}[\text{H}_2\text{A}]$ are of the same order of magnitude.

It has been shown that the binding of Cu(II) to histidine or imidazole has no significant effect on the catalytic activity of Cu(II),[4] as long as not all the coordination sites of copper are blocked by these bases. Hence, k_{3a} is not expected to be affected by binding to the polyelectrolyte[21] and the increase in R on binding of Cu(II) to polyhistidine is due to an effect on the preequilibrium.

The values of k_1 and k_{1a} are expected to be comparable for reaction 1 and 1a as these are specific rate constants for the replacement of the same ligand by the same H_2A.[21,22] As both k_1 and k_3 do not undergo appreciable changes upon binding of the copper to the polyelectrolyte, it must be concluded that the difference in the values of k_{obsd} as well as in the kinetic behavior of PLH-bound copper is due to a change in the $k_2[\text{H}^+]$ term, namely $k_{2a}[\text{H}^+] < k_2[\text{H}^+]$.

k_{2a} may be somewhat smaller than k_2 owing to the electrostatic repulsion of cations by the positively charged polyelectrolyte.[23] Moreover, the concentration of H^+ ions in the close vicinity of the positively charged polyelectrolyte is expected to be significantly lower than in the bulk of the solution.[24] Both these kinetic and thermodynamic effects will result in $k_{2a}\cdot[\text{H}^+] < k_2[\text{H}^+]$ leading to $(k_{1a}k_{3a}/k_2[\text{H}^+]_{\text{PLH}}) > (k_1k_3/k_2[\text{H}^+]_{\text{bulk}})$. Furthermore, the decrease in $k_{2a}[\text{H}^+]_{\text{PLH}}$ makes this term comparable with $k_{1a}[\text{H}_2\text{A}]$ at relatively low substrate concentration, leading to Michaelis–Menten kinetics.

Assuming that the inequality $k_{2a}[\text{H}^+]_{\text{PLH}} < k_2[\text{H}^+]_{\text{bulk}}$ is mainly due to the change in the average local concentration of H^+, one may estimate the electrostatic potential of the polyelectrolyte.[24] This can be done quantitatively only for a case where $R \sim 1/[\text{H}^+]$, *i.e.*, where $k_{2a}[\text{H}^+] >> (k_3 + k_1[\text{HA}])$. The kinetic behavior of the PLH–Cu(II)-catalyzed oxidation of homogentisate ions fulfills this requirement. From the difference in k_{obsd} of Cu(II) and PLH–Cu(II)-catalyzed oxidation of homogentisate ions, an electrostatic potential of 135 mv may be calculated; this value is in good agreement with electrostatic potentials found or calculated for other polyelectrolytes in aqueous solution.[24]

If the H_2A is negatively charged, its concentration in the vicinity of the positively charged polyelectrolyte will be greater than within the bulk of the solution.[23] This will lead to a further increase in the steady-state concentration of the PLH–Cu(II)–AH complex. The lower values of K_m for ascorbate and homogentisate ions as compared with hydroquinone may be explained by this electrostatic effect. On the other hand, positively charged substrates are repelled by PLH and the copper-catalyzed reaction is almost completely inhibited.

Electrolytes at high concentrations disturb the ionic distribution in the close environment of the polyelectrolyte and abolish the gradient of H^+ and H_2A^- between the polyelectrolyte and the bulk of solution. Under the latter conditions the polyhistidine-bound copper will approach and reach the normal catalytic efficiency of copper ions at the given pH. The addition of inert electrolytes will not, however, completely abolish the repulsion of positively charged substrates and even at high ionic strengths the electrostatic potential of the polyelectrolyte will not vanish completely.[24] Thus, the polyhistidine-bound Cu(II), in contrast to "monomeric" Cu(II), will remain less available to positively charged reductants even in the presence of relatively high concentrations of electrolytes.

The positive charge on the polyelectrolyte is neutralized with the increase of pH. The specific effect of polyhistidine on Cu(II) catalysis will, therefore, diminish with decreasing acidity, and the rate of oxidation may eventually reach the value of "monomeric" Cu(II) at the given elevated pH (Figure 4).[23]

In conclusion, it may be stated that *formally*, from the standpoint of reaction kinetics, the polyelectrolyte behaves as a second phase in which the concentration of reactants is different from those in the bulk of solution. The distribution coefficients which determine the relative concentrations of the reactants in the two phases depend primarily on electrostatic parameters. In other words, owing to electrostatic factors, one encounters heterogeneous kinetics in a homogeneous medium in our polyelectrolyte systems.

The kinetic behavior of the PLH–Cu(II)-catalyzed oxidation reactions are reminiscent of some enzymic oxidase processes. The similarity between the two types of catalysts is even greater if one considers the fact that copper bound to imidazole groups is likely to be part of the active site of some oxidases. This similarity is, however, rather formal as the products of reduction of oxygen are different for the two types of reactions: H_2O is produced in the oxidase reactions whereas PLH–Cu(II) yields H_2O_2 in a stoichiometric yield. Although the mechanisms of reaction of enzymic oxidase and of PLH–Cu(II) are evidently different, the specific effect of a polyelectrolyte on the pattern of catalytic reactions involving charged species, demonstrated in this study, may perhaps contribute to certain enzymic reactions.

(21) The comparability of k_{obsd} for the aquoacetato Cu(II) and Cu(II) in the presence of histidine or imidazole implies that k_1k_3/k_2 is similar in the two cases. As k_1 and k_2, which are the dissociative rate constants leading to ligand exchange,[22] are not appreciably affected by the presence of different ligands at coordination sites not involved in the exchange process,[22] the comparability of k_{obsd} implies that the values of k_3 and k_{3a} are of similar magnitude. k_3 may, however, be affected by complexants in the special case of ligands which form π bonds with copper; these ligands including azide, dipyridyl, or phenanthroline were shown to enhance the catalytic oxidation by Cu(II) to some extent.[2]

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