Interaction between Binuclear Copper(I) Complexes and the Dioxygen Molecule Investigated by Electrochemical Methods

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Electrochemistry, Binuclear Copper(II) Complexes, Copper(I), Dioxygen

The cyclic voltammograms of some binuclear copper(II) compounds with binucleating ligands where two molecules of tridentate ligand, N,N-bis(benzimidazol-2-ylmethyl)amine are linked by several alkyl chains, were measured under both argon and dioxygen. The results demonstrate that the binuclear copper(I) species produced by electrochemical reduction exhibit high reactivity towards dioxygen, while the reaction of the corresponding mononuclear species with oxygen is very slow. Thin-layer coulometry ([binuclear copper(I)]/[O_2] = 0.47–10.6) revealed that two molecules of the binuclear copper(I) species react with one molecule of dioxygen.

1. Introduction

Dopamine β-hydroxylase (DβM) is a copper-containing enzyme catalyzing the hydroxylation of dopamine to norepinephrine concomitant with a two-electron oxidation of ascorbic acid. The number of copper ions constituting an active site and the role played by enzyme-bound copper in the redox events of catalysis have been major questions in understanding this enzyme’s function [1]. In 1984, Klinman et al. have demonstrated that two copper ions per subunit are required in DβM turnover and in mechanism-based inactivation [2]. However, at present there is no spectroscopic evidence for significant magnetic interaction between copper centers in DβM, either in the resting form of the enzyme or in the enzyme-product complex, which is greatly different from species observed for the usual Type III copper enzymes [3]. This observation raises a central question regarding the DβM mechanism and structure; how can two copper ions, physically too insulated from one another to prevent magnetic interaction, constitute a site for the binding and subsequent activation of dioxygen? Stewart and Klinman suggested separate functions of two copper ions; one copper is proposed to be at the core of a “reductant site”, where ascorbate binds and delivers one electron at a time, while the second copper at a distance greater 4 Å is taken as the “active site” copper where dioxygen binding and activation take place [4].

In a series of known copper coordination complexes and in the protein tyrosinase and in heme-cyanin, the two-electron reduction of dioxygen to (metal-)peroxide involves binuclear copper compounds where both metal centers coordinate to O_2 [3]. Mononuclear copper complexes which catalyze oxidation of organic substrates have not been adequately investigated [5], and for this reason the mechanism proposed by Stewart and Klinman for the activation of dioxygen by mononuclear copper is unprecedented. Therefore more detailed investigations on the reaction between copper(I) compounds and dioxygen are required. Many reports have already been published on this problem [6]. It appears to be difficult to control the concentration of dioxygen in the reaction mixture under the experimental conditions previously used, making the study on the stoichiometry between copper ions and dioxygen to be critical. In this study we have investigated the reaction between copper(I) compounds, obtained by electrochemical reduction, and dioxygen in dmso (dimethylsulphoxide) solution. In our experimental set-up we can control the concentration of dioxygen in the reaction mixture, and thus our systems are favorable for the investigation on the stoichiometry involved.

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2. Materials and Methods

Materials

The ligands used in this study are illustrated below, and were obtained by published methods. The ligands (L - R) and (L - n) represent mononucleating [7] and binucleating [8] ligands, respectively. The copper(II) compounds with these ligands were prepared according to literature methods.

(I) Mononuclear complexes:

\[ \text{[Cu}(L - R)(\text{NO}_3)]\text{NO}_3, \quad R = \text{ethyl, } n\text{-propyl, } \tau\text{-butyl, benzyl.} \]

(II) Binuclear complexes:

\[ \text{[Cu}_2(L - n)(\text{NO}_3)_2]^2\] *.

Cyclic voltammogram (CV) measurements under Ar. The CV of the compounds were measured in the usual manner: in dmso, for 0.1 M tetra-(n-butyl)ammonium tetrafluoroborate as supporting electrolyte, and 0.0005 - 0.005 M metal complex. The potential was referenced to saturated sodium chloride calomel electrode (SSCE) at 25 °C. Ferrocene was used as a standard in determining the number of electrons participating in the electrochemical reactions.

CV measurements in the presence of O₂ molecule. Dioxygen gas or dry air was bubbled into the reaction mixture for 20 min at 25 °C, and after this CV measurements were carried out as described above. The concentration of dioxygen in the reaction mixture (in dmso, 25 °C) was evaluated as 2.1 and 0.47 mM for the solutions saturated by dioxygen or dry air, respectively, based on the work reported by Sawyer et al. [9].

Thin-layer coulometry and thin-layer CV measurements. The thin-layer coulometry and thin-layer CV were done according to published method [10]. The experimental conditions are summarized below.

Thin-layer coulometry in solution with [O₂] of 2.1 mM

\[ \text{[Cu}(L - \text{Et})(\text{NO}_3)]^+ \quad 1 \text{ mM} \]
\[ \text{[Cu}_2(L - 6)(\text{NO}_3)_2]^2+ \quad 0.5, 1, 2, 4 \text{ mM} \]
\[ \text{[Cu}_2(L - \text{px})(\text{NO}_3)_2]^2+ \quad 1 \text{ mM} \]

Thin-layer CV in solutions with [O₂] of 0.47 mM

\[ \text{[Cu}_2(L - 6)(\text{NO}_3)_2]^2+ \quad 2.5, 3.0, 3.5, 4.0, 5.0 \text{ mM} \]

3. Results and Discussion

ESR Spectral features of copper compounds

The binuclear copper(II) compounds used in this study are ESR-detectable as shown in Fig. 1. The spectra highly depend on the alkyl chain used [8]. This may be due to the presence of two separated copper(II) centers, the distance between two

![Fig. 1. ESR spectra (X-band, 77 K).
A: [Cu(L - Et)(NO₃)]NO₃ (in methanol);
B: Cu₂(L - 6)(NO₃)$_2$ (in dmf).](image-url)
copper ions being controlled by the alkyl chains. ESR parameters [7] of mononuclear complexes such as \([\text{Cu}(L-\text{Et})(\text{NO}_3)]^+\), \(g_L = 2.28\) and \(g_z = 2.06\), are very similar to those observed for \(D/M\) \((g_L = 2.27, g_z = 2.05)\) [11], indicating that the coordination environment around the copper ion of the present binuclear compounds is very similar to that in \(D/M\).

**Electrochemical properties**

The representative semi-infinite CV of the mononuclear and binuclear copper(II) compounds in dmso under Ar are shown in Figs. 2A and 3A. The electrochemical data of all the compounds examined in this study are summarized in Table I. A quasi-reversible reduction is observed under Ar for every copper(II) compound at 0.06–0.13 V (vs. SSCE). The thin-layer coulometry reveals that the numbers of electrons involved in the reduction process are one and two for the mononuclear and binuclear compounds, respectively. The values of

<table>
<thead>
<tr>
<th>Complexes</th>
<th>(E^{°}/V) vs. SSCE</th>
<th>(\Delta E/mV)</th>
<th>(I_{pc}/S^c)</th>
<th>(i_{pa}/i_{pc})</th>
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<tbody>
<tr>
<td>A: Mononuclear complexes</td>
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<tr>
<td>a) (O_2) free</td>
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<tr>
<td>([\text{Cu}(L-\text{Bz})(\text{NO}_3)]^+) &amp; 0.06 &amp; 100 &amp; 277 &amp; 1.2</td>
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<tr>
<td>([\text{Cu}(L-\text{Et})(\text{NO}_3)]^+) &amp; 0.03 &amp; 80 &amp; 300 &amp; 1.2</td>
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<tr>
<td>([\text{Cu}(L-\text{n-Pr})(\text{NO}_3)]^+) &amp; 0.04 &amp; 90 &amp; 338 &amp; 1.2</td>
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<tr>
<td>([\text{Cu}(L-\text{t-Bu})(\text{NO}_3)]^+) &amp; 0.13 &amp; 125 &amp; 255 &amp; 1.3</td>
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<td>b) (O_2) saturated</td>
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<tr>
<td>([\text{Cu}(L-\text{Bz})(\text{NO}_3)]^+) &amp; 0.06 &amp; 100 &amp; 286 &amp; 1.1</td>
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<tr>
<td>([\text{Cu}(L-\text{Et})(\text{NO}_3)]^+) &amp; 0.03 &amp; 100 &amp; 309 &amp; 1.0</td>
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<tr>
<td>([\text{Cu}(L-\text{n-Pr})(\text{NO}_3)]^+) &amp; 0.04 &amp; 100 &amp; 344 &amp; 1.0</td>
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<tr>
<td>([\text{Cu}(L-\text{t-Bu})(\text{NO}_3)]^+) &amp; 0.14 &amp; 175 &amp; 250 &amp; 1.0</td>
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<td>B: Binuclear complexes</td>
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<tr>
<td>a) (O_2) free</td>
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<tr>
<td>([\text{Cu}_2(L-3)(\text{NO}_3)_2]^{2+}) &amp; 0.02 &amp; 160 &amp; 411 &amp; 1.4</td>
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<tr>
<td>([\text{Cu}_2(L-4)(\text{NO}_3)_2]^{2+}) &amp; 0.03 &amp; 140 &amp; 326 &amp; 1.4</td>
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<tr>
<td>([\text{Cu}_2(L-6)(\text{NO}_3)_2]^{2+}) &amp; 0.04 &amp; 170 &amp; 344 &amp; 1.3</td>
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<tr>
<td>([\text{Cu}_2(L-\text{px})(\text{NO}_3)_2]^{2+}) &amp; 0.08 &amp; 250 &amp; 320 &amp; 1.2</td>
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<tr>
<td>([\text{Cu}_2(L-3-\text{OH})(\text{NO}_3)_2]^{2+}) &amp; 0.06 &amp; 180 &amp; 383 &amp; 1.3</td>
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<td>b) (O_2) saturated</td>
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<tr>
<td>([\text{Cu}_2(L-3)(\text{NO}_3)_2]^{2+}) &amp; (–0.06) &amp; – &amp; 523 &amp; –</td>
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<tr>
<td>([\text{Cu}_2(L-4)(\text{NO}_3)_2]^{2+}) &amp; (–0.07) &amp; – &amp; 416 &amp; e</td>
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<tr>
<td>([\text{Cu}_2(L-6)(\text{NO}_3)_2]^{2+}) &amp; (–0.02) &amp; – &amp; 443 &amp; –</td>
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<td>([\text{Cu}_2(L-\text{px})(\text{NO}_3)_2]^{2+}) &amp; (–0.10) &amp; – &amp; 358 &amp; f</td>
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<tr>
<td>([\text{Cu}_2(L-3-\text{OH})(\text{NO}_3)_2]^{2+}) &amp; (–0.02) &amp; – &amp; 488 &amp; –</td>
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Table I. Cyclic voltammetric data (in dmso, scan rate 100 mV/s, 25 °C, metal complex, 1 mM).

\(^a\) \(E^{°} = (E_{pa} + E_{pc})/2\). If no reverse peak is observed, the peak potential \(E_{pc}\) at 0.1 V/s is given in parentheses; \(^b\) \(\Delta E = [E_{pc} - E_{pa}];\)
\(^c\) \(S = \mu A \times V^{1/2} \times \text{cm}^{-2} \times \text{mM}^{-1}\), peak current parameters; \(^d\) peak current ratio; \(^e\) the oxidation peaks are observed at a faster scan rate (1 V/s, 0.2 V/s).
the peak current ratio \((i_{pa}/i_{pc})\) of these reduction processes are slightly larger than unity, and increase with the increasing scan rate, showing that the reduction products of these compounds are weakly adsorbed. Since the value of \(i_{pa}/i_{pc}\) approaches unity with decreasing scan rate, the reduction of these compounds is essentially reversible. Thus, the reduction processes of these compounds are written as follows.

(i) Mononuclear compound

\[
[Cu^{II}(L-\text{R})(NO_3)]^+ + e^- \rightleftharpoons [Cu^{III}(L-\text{R})(NO_3)]
\]

(ii) Binuclear compound

\[
[Cu^{II}_2(L-n)(NO_3)_2]^{2+} \rightleftharpoons 2[Cu^{III}(L-n)(NO_3)]
\]

The binuclear copper(II) compounds studied in this work exhibit a single two-electron reduction and the formal potentials are comparable to those of the corresponding mononuclear ones. Furthermore, the voltammetric behaviour of the binuclear compounds is very similar to that of the mononuclear compounds. Based on these results, it is concluded that the binuclear copper(II) compounds used in this study have two identical and independent redox centers which are equivalent to the mononuclear compound.

Figs. 2B and 3B show the representative semi-infinite CV of the copper(II) compounds in the presence of dioxygen. Because the formal potential of the \(O_2/O_2^-\) redox couple is \(-0.72\) V (vs. SSCE) in DMSO, \(O_2\) is electrochemically inactive in the region (around 0 V) where the copper(II) complexes used in this study are reduced to copper(I) species. The voltammetric behaviour of the mononuclear compounds under \(O_2\) is little different from that under Ar at a scan rate 0.1 V/s (cf. Fig. 2). On the other hand, the presence of \(O_2\) caused drastic changes in the semi-infinite CV of all the binuclear compounds; i.e., the reduction process of the binuclear compounds becomes irreversible under \(O_2\); the value of \(i_{pa}/i_{pc}\) decreases to almost zero, as shown in the figures (cf. also Table I). This indicates that the binuclear copper(I) species produced by the electrochemical reduction reacts readily with \(O_2\), although the reaction between mononuclear copper(I) species and dioxygen is very slow. The above results strongly imply that two copper ions should participate in the reaction and activation of dioxygen in DMSO. The decrease of \(i_{pa}/i_{pc}\) to zero was observed for a wide range of copper ion to dioxygen ratios \([\text{copper(II)}]/[O_2] = 0.47-1.9\) and for all scan rates \((0.1-1.0\ \text{V/sec})\), except for the \((L-\text{px})\) system. In the case of \([Cu^{II}_2(L-\text{px})(NO_3)_2]^{2+}\), a partially reversible wave is observed for the semi-infinite CV at a scan rate of 1 V/s as shown in Fig. 4B. This indicates that the binuclear copper(I) species with \((L-\text{px})\) produced by the electrochemical reduction is less reactive to-
wards O_2 than with (L-4) and (L-6), although the electrochemical behaviour of [Cu^{II}_{2}(L-px)(NO_3)_2]^{2+} under Ar is very similar to that of other binuclear compounds. This suggests that the reactivity of the binuclear copper(I) species towards O_2 depends on the steric configuration of the two copper(I) ions.

In order to get more information on the reaction between binuclear copper(I) species and dioxygen, further investigations were carried out employing thin-layer CV and coulometry. In the absence of O_2, the thin-layer CV (Fig. 5A) of [Cu^{II}_{2}(L-6)(NO_3)_2]^{2+} exhibits a reversible reduction, and i_{pa}/i_{pc} is almost unity even at a very slow scan rate (0.04 V/sec). In the presence of O_2 (0.47 mM, saturated by dry air), i_{pa} is smaller than that observed under Ar as shown in Fig. 5B. If we assume that the decrease of i_{pa} in the presence of O_2 can be attributed to the decrease of the produced copper(I) species due to the oxidation by the reaction with O_2 (reaction (4)), we can determine the value of X in (4).

\[
X[Cu^{II}_{2}(L-6)(NO_3)_2] + O_2 \rightarrow X[Cu^{II}_{2}(L-6)(NO_3)_2] + O_2^{2-} \quad (4)
\]

by evaluation the i_{pa} value with various concentrations of copper(I) species under a constant O_2 concentration. The results (O_2 concentration, 0.47 mM; copper(II) concentration, 2-5 mM) show that X is 2.0 ± 0.4 (average of five determinations; the error being estimated from the standard deviation at the 95% confidence level), indicating that two molecules of binuclear copper(I) species react with one molecule of dioxygen. The thin-layer coulometry study revealed that the number of electrons transferred during the reduction process of [Cu^{II}_{2}(L-6)(NO_3)_2]^{2+} is determined as three per one binuclear copper(II) complex even in the presence of excess O_2. The three-electron reduction was also observed for the corresponding (L-px) compound. Based on these results, it seems likely that the reduction of binuclear copper(II) compounds in the presence of O_2 may proceed as follows (total reaction, (9)).

\[
[Cu^{II}_{2}(L-n)]^{2+} + 2e^- \rightarrow [Cu^{II}_{2}(L-n)] \quad (5)
\]

\[
[Cu^{II}_{2}(L-n)] + O_2 \rightarrow [Cu^{II}_{2}(L-n)O_2] \iff [Cu^{II}_{2}(L-n)O_2^{2-}] \quad (6)
\]

\[
[Cu^{II}_{2}(L-n)O_2^{2-}] + [Cu^{II}_{2}(L-n)] \rightarrow [Cu^{II}_{2}(L-n)O_2^{2-}Cu^{II}_{2}(L-n)] \quad (7)
\]

\[
[Cu^{II}_{2}(L-n)O_2^{2-}Cu^{II}_{2}(L-n)] + 2e^- \rightarrow 2[Cu^{II}_{2}(L-n)] + O_2^{2-} \quad (8)
\]

\[
2[Cu^{II}_{2}(L-n)] + O_2 + 6e^- \rightarrow 2[Cu^{II}_{2}(L-n)] + O_2^{2-} \quad (9)
\]

This implies that a (1:1) dioxygen adduct derived from binuclear copper(I) species (cf. the equation (6) and Fig. 6A) may exhibit high electrophilicity, leading to a facile (2:1) complex formation (equation (7); cf. Fig. 6B) under our experimental conditions ([binuclear copper(I)]/[O_2] = 0.47-10.7).

![Fig. 6. Assumed structure for the A: (1:1) peroxide adduct, and B: (2:1) adducts.](image)

**Explanation for facile (2:1) complex formation**

Recently Nishida et al. [12] have demonstrated that the peroxide ion in several binuclear copper(II) and iron(III) compounds exhibits high reactivity towards several organic compounds, and have elucidated its reactivity in terms of the Frontier Orbital Theory. The high reactivity of the peroxide ion in these binuclear compounds has been attributed to the presence of an unoccupied orbital at rather lower energy, which is composed
of a π-orbital of the peroxide ion and a metal d-orbital; the presence of such an orbital is supported by the recent theoretical calculation of Solomon et al. [13] (see Fig. 7). This orbital can interact with the occupied orbitals of the substrate in the region indicated by the arrow in Fig. 7. It is generally known that the compounds possessing unoccupied orbitals with lower energy exhibit high electrophilicity [14]. This may give a reasonable explanation for the high electrophilicity of a (1:1) dioxygen adduct of binuclear copper(I) species [12] and thus facile (2:1) complex formation (cf. Fig. 6).

We are thankful for the supports from the Inamori Foundation and Grant-in-Aid for Scientific Research No. 02403012 from the Ministry of Education, Science and Culture.

Fig. 7. MO levels of a binuclear copper(II) peroxide adduct [13].