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Tetracopper Assembly Complexes Comprised of One Dimetallic Core and Two Monometallic Auxiliaries: Intramolecular Electron-Transfer Relevant to Multicopper Oxidases

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Two tetracopper assembly complexes, comprised of one dimetallic di(3-iminomethylsalicylato)dicopper(II) core and two monometallic copper(II) auxiliaries attached to the imino nitrogens of the dinuclear core through an alkane chain, have been prepared. $[Cu_4(L^1)](PF_6)_4 \cdot 2CH_3CN \cdot 3H_2O$ (**1**) has di(2-pyridylmethyl)aminecopper(II) as the monometallic auxiliary, and $[Cu_4(L^2)](ClO_4)_4 \cdot CH_3OH$ (**2**) has 1,4,8,11-tetraazacyclotetradecanecopper(II) as the auxiliary. Assembly **1** in acetonitrile shows a two-electron reduction at −0.08 V (vs SCE) followed by a one-electron reduction at −0.42 V. Together with EPR studies for electrolyzed solutions, it is shown that the two monometallic auxiliaries are reduced at −0.08 V, followed by an intramolecular electron transfer from one of the reduced auxiliaries to the dimetallic core and by the second reduction at the resulting monometallic Cu^{II} center at −0.42 V: $\{Cu(II)-Cu_2(II,II)-Cu(II)\}/\{Cu(I)-Cu_2(II,II)-Cu(I)\} \rightarrow \{Cu(I)-Cu_2(I,II)-Cu(II)\}/\{Cu(I)-Cu_2(I,II)-Cu(I)\}$. The CV of **2** in DMSO shows two couples at −0.68 and −0.99 V attributable to the stepwise reductions: $\{Cu(II)-Cu_2(II,II)-Cu(II)\}/\{Cu(II)-Cu_2(I,II)-Cu(II)\}/\{Cu(I)-Cu_2(I,II)-Cu(II)\}$. Assembly **1** is reduced with ascorbic acid to the $\{Cu(I)-Cu_2(I,II)-Cu(I)\}$ species, whereas **2** is not reduced with ascorbic acid. The relevance of the intramolecular electron transfer observed for **1** to multicopper oxidases is discussed.

Introduction

It is known[1−4] that multicopper oxidases such as laccase, ceruloplasmin, and ascorbate oxidase contain all three types of copper (monometallic type 1, monometallic type 2, and dimetallic type 3) and catalyze the four-electron reduction of dioxygen to water with concomitant oxidation of substrate. Laccase is the simplest of this family and contains four copper atoms from each of the three types.[5] Ascorbate oxidase has two subunits[6] and can be described as a dimer of a laccase site with a total of eight copper atoms. X-ray crystallography for the oxidized form of ascorbate oxidase from green zucchini squash has been performed.[7] The type 2 and type 3 copper atoms are in close proximity, forming a triangular cluster, while the type 1 site, closely analogous to that of plastocyanin, is separated by 12.2 Å from the type 3 center (Chart 1). Ceruloplasmin has a similar triangular array of type 2 and type 3 copper, and type 1 copper is separated by 12−13 Å from the type 3 center.[8] In this enzyme two additional type 1 coppers exist that are largely isolated from the triangular cluster. The type 2 depleted derivative of laccase does not react with dioxygen,[9] whereas the laccase derivative having a redox-inactive Hg^{2+} in place of the type 1 copper reacts with dioxygen.[10,11] These facts indicate that the triangular cluster of type 2 and type 3 is the minimum unit for dioxygen reduction.[12] Thus, first generation model

Chart 1. Triangular (Type 2)/(Type 3) Cluster of Ascorbate Oxidase

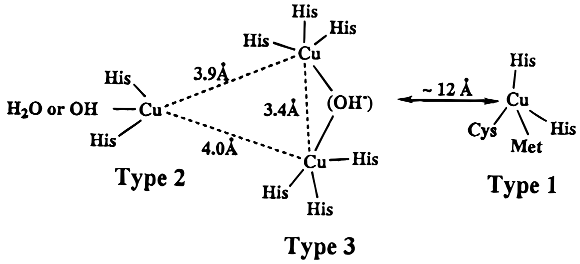

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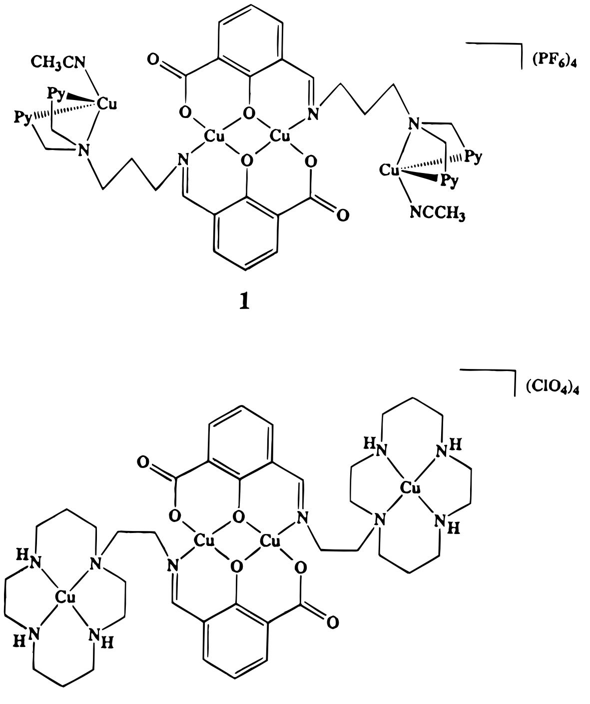
Chart 2. Chemical Structures of the Tetracopper Assembly Complexes $[Cu_4(L^1)](PF_6)_2 \cdot 2CH_3CN \cdot 3H_2O$ (1) and $[Cu_4(L^2)](ClO_4)_2 \cdot CH_3OH$ (2)



1

2

studies of multicopper oxidases have been devoted to replicating the triangular cluster of type 2 and type 3.[13−15]

The function of type 1 copper is to receive an electron from substrate and transfer the electron, in conjunction with type 2 copper, to the type 3 center, which is the site of dioxygen interaction.[16] In this context, tetracopper assembly complexes comprised of one dimetallic core and two monometallic auxiliaries are of great interest in the interplay of the copper ions in the redox process. In this study, two tetracopper assembly complexes, $[Cu_4(L^1)](PF_6)_4 \cdot 2CH_3CN \cdot 3H_2O$ (**1**) and $[Cu_4(L^2)](ClO_4)_4 \cdot CH_3OH$ (**2**) (Chart 2), have been prepared. They are comprised of one dimetallic bis(3-iminomethylsalicylato)dicopper(II) core and two monometallic copper(II) auxiliaries that are connected to the imino nitrogens of the dimetallic core through an alkane chain. Assembly **1** has di(2-pyridylmethyl)aminecopper(II) and **2** has 1,4,8,11-tetraazacyclotetradecanecopper(II) as the monometallic auxiliary. In this report, the main focus is placed on the electrochemical and chemical properties of **1** that shows an intramolecular electron transfer from the reduced monometallic auxiliary to the dimetallic core.

Experimental Section

Material. Unless otherwise stated all chemicals were purchased from commercial sources and used without further purification. Solvents were dried and purified by standard methods. 3-Formylsalicylic acid[17] and *N*-(*p*-toluenesulfonyl)aziridine[18] were prepared by the literature methods.

Preparation. *N*,*N*-Di(2-pyridylmethyl)-1,3-propanediamine (dpytn). Acrylonitrile (0.69 g, 13 mmol) was dropwise added to a solution of di(2-pyridylmethyl)amine (1.99 g, 10 mmol) in methanol at room temperature, and the mixture was heated at 50 °C for 72 h. Unreacted acrylonitrile was separated by distillation under a reduced pressure to give crude *N*,*N*-di(2-pyridylmethyl)-3-aminopropionitrile (ca. 2.1 g). It was dissolved in methanol, added to a slurry of Raney nickel (0.3 g) in water (1 cm^3), and reduced with $NaBH_4$ (4.0 g) dissolved in 4% NaOH solution (100 cm^3) at ~60 °C. The resulting oily *N*,*N*-di(2-pyridylmethyl)-1,3-propanediamine (dpytn) was separated by extraction with dichloromethane (200 cm^3) and used for the synthesis of assembly **1** without further purification. Yield: 2.6 g.

1-(2-Aminoethyl)-1,4,8,11-tetraazacyclotetradecane Pentahydrobromide Dihydrate (aetacd·5HBr·2H$_2$O). To a boiling solution of 1,4,8,11-tetraazacyclotetradecane (8.0 g, 0.04 mol) in acetonitrile (1000 cm^3) was dropwise added a solution of *N*-(*p*-toluenesulfonyl)aziridine (7.88 g, 0.04 mol) in acetonitrile (500 cm^3). The mixture was heated at reflux temperature for 3 days and evaporated to dryness to give crude 1-[2-(*N*-*p*-toluenesulfonyl)aminoethyl]-1,4,8,11-tetraazacyclotetradecane (14.4 g). It was suspended in a mixture of acetic acid (260 g) and hydrobromic acid (47%, 260 g), and the whole mixture was heated at the reflux temperature for 40 h. The reaction mixture was evaporated to dryness, and the resulting product was recrystallized from ethanol as colorless needles. Yield: 78%. Anal. Found: C, 21.21; H, 5.55; N, 9.99. Calcd for $C_{12}H_{38}Br_5N_5O_2$: C, 21.07; H, 5.60; N, 10.24.

$[Cu_4(L^1)](PF_6)_4 \cdot 2CH_3CN \cdot 3H_2O$ (1). A solution of dpytn (248 mg, 1 mmol) in methanol (10 cm^3) was added to a suspension of di(3-formylsalicylato)dicopper(II) $[Cu_2(fsac)_2]$[19] (0.228 g, 0.5 mmol) in methanol (10 cm^3), and the mixture was refluxed for 1 h. A methanol solution (10 cm^3) of copper(II) nitrate trihydrate (242 mg, 1 mmol) was added, and the mixture was refluxed for 1 h and evaporated to dryness. The residue was triturated with an acetonitrile solution of ammonium hexafluorophosphate (excess), and any insoluble material (NH_4NO_3) was separated by filtration. When ether was diffused into the filtrate, assembly **2** was deposited as bluish green microcrystals. Yield: 0.30 g (35%). Anal. Found: C, 33.93; H, 3.23; N, 7.47; Cu, 14.38. Calcd for $C_{50}H_{56}Cu_4F_{24}N_{10}O_9P_4$: C, 33.83; H, 3.18; N, 7.89; Cu, 14.32. μ_{eff} per molecule: 2.92 μ_B at 290 K. Selected IR [ν/cm^{-1}] using KBr disks: 1620, 1600, 1560, 1540, 840. Molar conductance [Λ_M/S cm^2 mol^{-1}] in CH_3CN: 400. UV−vis [λ/nm (ϵ/M^{-1} cm^{-1})] in CH_3CN: 335 (15 700), 653 (420).

$[Cu_4(L^2)](ClO_4)_2 \cdot CH_3OH$ (2). An aqueous solution of aetacd·5HBr·2H$_2$O (1.03 g, 1.5 mmol) was made alkaline by adding an aqueous solution of KOH, and the liberated amine was separated by chloroform extraction. A methanol solution of aetacd was added to a suspension of $[Cu_2(fsac)_2]$ (0.228 g, 0.5 mmol) in methanol, and the mixture was refluxed for 2 h. Any insoluble precipitate was separated by filtration, then a methanol solution of copper(II) perchlorate hexahydrate (0.556 g, 1.5 mmol) was added to the filtrate, and the mixture was refluxed overnight to result in the precipitation of purple microcrystals. Yield: 0.61 g (85%). Anal. Found: C, 33.69; H, 4.31; N, 9.52; Cu, 17.22. Calcd for $C_{41}H_{66}Cl_4Cu_4N_{10}O_{23}$: C, 33.67; H, 4.54; N, 9.57; Cu, 17.37. μ_{eff} per molecule: 2.78 μ_B at 290 K. Selected IR [ν/cm^{-1}] using KBr disks: 1620, 1600, 1140, 1110, 1090, 620. Molar conductance [Λ_M/S cm^2 mol^{-1}] in DMSO: 110. UV−vis [λ/nm (ϵ/M^{-1} cm^{-1})] in DMSO: 337 (14 000), 605 (440).

Reference Complexes. Di(3-*N*-benzyliminomethylsalicylato)dicopper(II) $[Cu_2(fsac\text{-}bz)_2]$ was obtained by the literature method[20] as a reference for the dimetallic core. Dichloro(*N*,*N*-di(2-pyridylmethyl)amine)copper(II), Cu(dpa)Cl$_2$, was obtained as a reference for the monometallic auxiliary for **1** by the reaction of dpa with copper(II) chloride.[21] 1,4,8,11-Tetraazacyclotetradecanecopper(II) perchlorate [Cu(cyclam)](ClO$_4$)$_2$ was prepared as a reference for the monometallic

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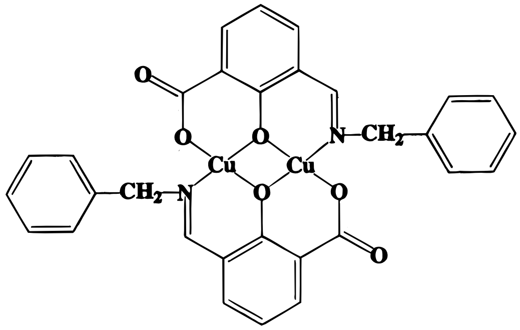
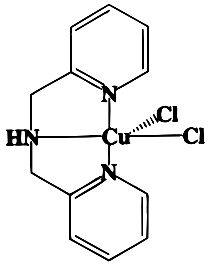
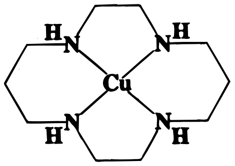
Chart 3. Chemical Structures of Reference Complexes



[Cu$_2$(fsac-bz)$_2$]



Cu(dpa)Cl$_2$



[Cu(cyclam)](ClO$_4$)$_2$

auxiliary for **2** by the literature method.[22] The chemical structures of the reference complexes are shown in Chart 3.

Physical Measurements. Elemental analyses of C, H, and N were obtained at the Elemental Analysis Service Center of Kyushu University. Copper analyses were made with a Shimadzu AA-680 atomic absorption/flame emission spectrophotometer. Infrared spectra were recorded on a JASCO IR-810 spectrometer using KBr disks. Molar conductances were measured on a DKK AOL-10 conductivity meter at 20 °C at $\sim 1 \times 10^{-3}$ M concentration. Electronic absorption spectra were recorded on a Shimadzu MPS-2000 spectrometer. Magnetic susceptibility measurements were performed on a Faraday balance in the temperature range of 80−300 K. The apparates were calibrated using $[Ni(en)_3]S_2O_3$.[23] Diamagnetic corrections for constituting atoms were made using Pascal's constants.[24] X-band EPR spectra were recorded on a JEOL JEX-FE3X spectrometer. Electrochemical data (CV and RDE) for **1** were obtained in acetonitrile on a RDE3 pine potentiostat, using a glassy carbon (GC) disk sealed in Teflon as the working electrode, a Pt wire as the counter electrode, and AgCl/Ag wire in DMF/TBACl (TBACl = tetra(*n*-butyl)ammonium chloride) as the quasi-reference electrode. The supporting electrolyte was tetra(*n*-butyl)ammonium hexafluorophosphate. The electrochemical data for **2** were obtained in DMSO on a BAS CV-50W electrochemical analyzer, using tetra(*n*-butyl)ammonium perchlorate (TBAP) as the supporting electrolyte (**Caution!** TBAP is explosive and should be handled with great care). The potentials of redox waves were referenced internally to the ferrocenium/ferrocene couple (+0.425 V vs SCE in acetonitrile and +0.363 V vs SCE in DMSO). Coulometric studies were performed on an apparatus comprising a HA-501 potentiostat/galvanostat, a HB-104 function generator, and a HF-201 coulomb/ampere hour meter from Hokuto Denko Ltd.

Results and Discussion

Synthesis and General Properties. Synthetic schemes for **1** and **2** are shown in Scheme 1. The FAB mass spectra for assembly **1** shows three dominant ion peaks centered around *m*/*z* = 1493, 1204, and 1059 that correspond to $\{Cu_4(L^1)(PF_6)_3\}^+$, $\{Cu_4(L^1)PF_6 + 2e^-\}^+$, and $\{Cu_4(L^1) + 3e^-\}^+$, respectively. Assembly **2** has an ion peak centered around *m*/*z* = 1333 that is attributable to $\{Cu_4(L^2)(ClO_4)_3\}^+$. The formyl ν(CO) vibration of $[Cu_2(fsac)_2]$ (1650 cm^{-1})[19] is replaced by the ν(C=N) vibration[20] near 1625 cm^{-1} in both **1** and **2**. The intense IR band at 840 cm^{-1} of **1** is characteristic of the PF_6^- ion. The perchlorate ν_3 mode of **2** splits into three (1140, 1110, and 1090 cm^{-1}), suggesting a weak coordination of the ion in the crystal.

The room-temperature magnetic moments of **1** and **2** (per four Cu atoms) are 2.92 and 2.78 μ_B, respectively, which decrease with decreasing temperature to reach plateau values of 2.61 and 2.45 μ_B, respectively, around 100 K. The plateau values correspond well to the spin-only value for two isolated spins (2.45 μ_B). The cryomagnetic property observed suggests that the dimetallic core and the monometallic auxiliaries are magnetically independent of each other, and a strong antiferromagnetic interaction operates within the dimetallic core to achieve a perfect coupling above 100 K. This is supported by our previous magnetic studies for di(3-*N*-alkyliminosalicylato)-dicopper(II) complexes[20] showing strong antiferro-magnetic coupling.

The molar conductance of **1** in acetonitrile is 400 S cm^2 mol^{-1}, and that of **2** in DMSO is 112 S cm^2 mol^{-1}, indicating that both assemblies behave as 4:1 electrolytes in each solvent.[25] Assembly **1** has a d−d band maximum at 653 nm (ϵ = 420 dm^3 mol^{-1} cm^{-1}) in acetonitrile. The visible spectrum of **2** in DMSO has a d−d band maximum at 605 nm (ϵ: 440 dm^3 mol^{-1} cm^{-1}). The intense band in the near-ultraviolet region (339 nm for **1** and 339 nm for **2**) can be assigned to a $\pi \rightarrow \pi^*$ transition of the azomethine linkage.[26,27]

Assembly **1** shows an axial pattern of EPR with $g_\parallel = 2.23$, $g_\perp = 2.04$, and $A_\parallel = 175 \times 10^{-4}$ cm^{-1} on frozen acetonitrile solution at liquid nitrogen temperature (see Figure 3a). The EPR spectrum is characteristic of an isolated Cu(II) having one unpaired electron on $d_{x^2-y^2}$ orbital (*x* and *y* axes being taken along donor atoms)[28] and can be attributed to the monometallic auxiliaries; the EPR parameters are compared to those for the monometallic reference Cu(dpa)Cl$_2$ ($g_\parallel = 2.21$, $g_\perp = 2.01$, and $A_\parallel = 199 \times 10^{-4}$ cm^{-1}), and the bimetallic core is EPR silent owing to a strong antiferromagnetic interaction.[19] The EPR spectrum of **2** in frozen DMSO solution also shows an axial pattern with $g_\parallel = 2.18$, $g_\perp = 2.06$, and $A_\parallel = 196 \times 10^{-4}$ cm^{-1}; the EPR parameters are compared to those of the monometallic reference [Cu(cyclam)](ClO$_4$)$_2$ ($g_\parallel = 2.18$, $g_\perp = 2.05$, and $A_\parallel = 206 \times 10^{-4}$ cm^{-1}). The large $g_\parallel$ value and small $A_\parallel$ value of **1**, relative to those of **2**, reflect a distorted configuration about

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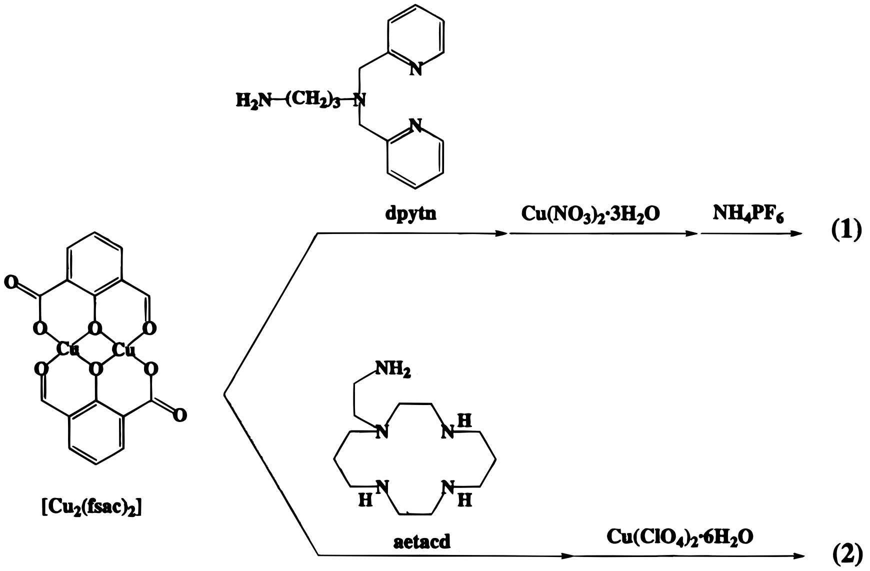
Scheme 1. Synthetic Schemes for 1 and 2



the auxiliary Cu(II). That is, the auxiliary Cu(II) of **1** can distort toward a tetrahedral structure whereas that of **2** is essentially planar.[23]

Electrochemistry. Prior to electrochemical studies of **1** and **2**, it is of value to know the redox behavior of each component of the assemblies. In DMSO, [Cu_2(fsac-benzyl)$_2$] (a reference of the dimetallic core), [Cu(dpa)Cl_2] (a reference for the monometallic auxiliary of **1**), and [Cu(cyclam)](ClO_4)$_2$ (a reference for the monometallic auxiliary of **2**) are reduced at −0.67, −0.26, and −0.98 V, respectively. Therefore, it is likely that the monometallic auxiliary is reduced prior to the dimetallic core in assembly **1**, whereas the dimetallic core is reduced prior to the monometallic auxiliary in **2**.

The cyclic voltammograms of assembly **1** measured in acetonitrile are given in Figure 1. A series of waves can be observed between 0 and −1.2 V, but their location and band shape vary somewhat with the number of scans and state of the electrode due to severe adsorption problems (see Figure 1a) and current intensity drops with each successive cycle (first cycle shown). The adsorption wave evident near −0.12 V can be avoided if the switching potential is limited to about −0.6 V, as shown in Figure 1b, and continuous cycling in this range led to reproducible redox waves. Thus, these first two waves can be discussed, but it is not profitable to discuss the significance of waves more negative than this. The open-circuit potential is about +0.30 V, so the two waves at −0.06 and −0.47 V are reduction processes.

To determine the number of electrons involved in the first (ca. −0.08 V) and the second (ca. −0.42 V) reduction steps, coulometric and rotating-disk electrode (RDE) experiments were performed. Coulometry at −0.4 V indicated the transfer of three electrons altogether. In RDE experiments, two reduction waves were observed at −0.08 and −0.42 V (Figure 2). The ratio of the two limiting currents clearly indicates that the first reduction involves two-electron transfer and the second reduction involves one-electron transfer.

Figure 3 shows EPR spectra of **1** (trace a) and its electrolyzed, frozen solutions (b and c). Trace b is the EPR spectrum for the three-electron reduced solution (electrolyzed at −0.35 V) that shows an EPR signal of $g'_{\parallel} = 2.27$, $g'_{\perp} = 1.98$, $A'_{\parallel} = 137 \times 10^{-4}$ cm^{-1}, and $A'_{\perp} = 82 \times 10^{-4}$ cm^{-1}. We have observed a similar EPR spectrum for the Cu_2(I,II) complex of [Cu_2(fsac-bz)$_2$] (electrolyzed at −0.76 V in DMSO). Our EPR studies clearly demonstrate that the three-electron reduced species is {Cu(I)−Cu_2(I,II)−Cu(I)} whose one unpaired electron in the dimetallic core is trapped on one copper at this temperature. It is known that seven-line hyperfine structure is observed for spin-delocalized Cu_2(I,II) in solution, but spin is usually trapped when measured in frozen solution to give the usual four-line structure.[29−31] At room temperature, the electrolyzed solution

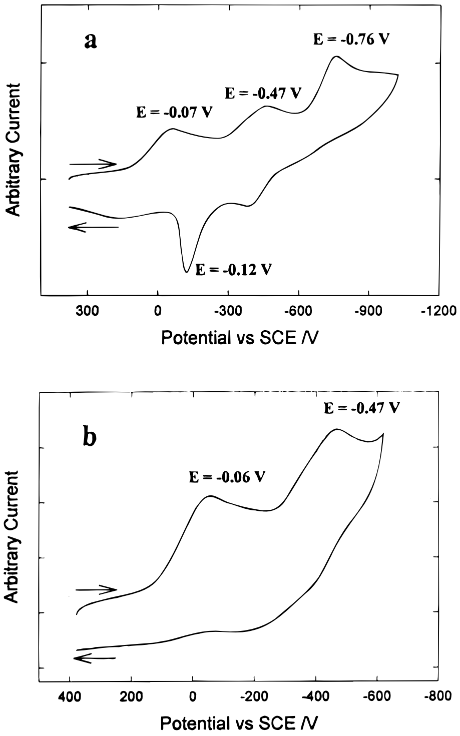


Figure 1. Cyclic voltammograms of **1** in acetonitrile: (a) the first scan in the range from +0.7 to −1.3 V, (b) in the range from +0.7 to −0.6 V. Conditions: GC working electrode, Pt wire counter electrode, AgCl/Ag quasi-reference electrode, TBAP (0.1 M) as the supporting electrolyte, scan rate 100 mV/s.

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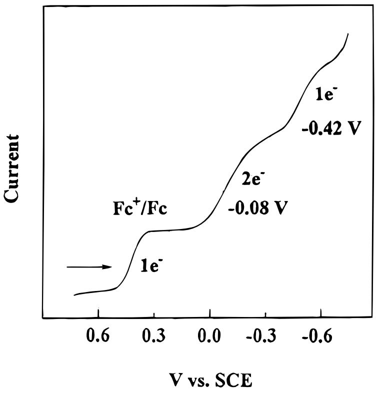


Figure 2. Polarogram of **1** measured on a RDE in acetonitrile. Conditions: GC disk working electrode, Pt wire counter electrode, AgCl/Ag wire quasi-counter electrode, spin velocity 378 rpm, scan rate 5 mV/s.

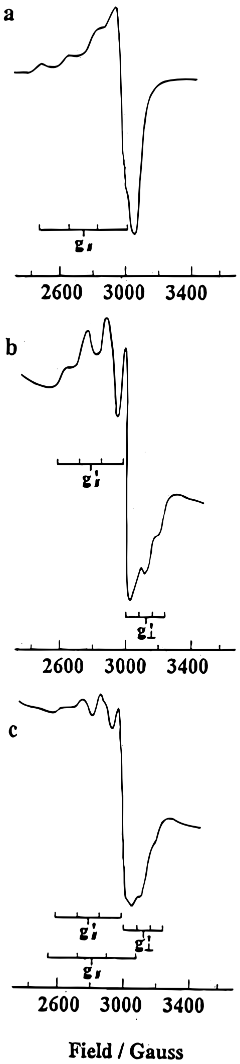


Figure 3. EPR spectra of **1** and its electrolyzed complexes in frozen acetonitrile solution: (a) assembly **1**, (b) three-electron reduced complex (electrolized at -0.42 V), and (c) two-electron reduced complex (electrolized at -0.08 V).

of **1** gave a broad EPR signal around $g = 2$, probably due to slow tangling of the assembly molecule in solution.

Trace c of Figure 3 is the EPR spectrum for the two-electron reduced solution of **1** (electrolyzed at -0.35 V). This can be regarded as the superpositon of two types of EPR attributable to the monometallic auxiliary ($g_\parallel = 2.23$, $g_\perp = 2.04$, and $A_\parallel = 175 \times 10^{-4}$ cm^{-1}) and to the spin-localized dimetallic Cu$_2$(I,II) ($g'_\parallel = 2.27$, $g'_\perp = 1.98$, $A'_\parallel = 137 \times 10^{-4}$ cm^{-1}, and $A'_\perp = 82 \times 10^{-4}$ cm^{-1}). Evidently, the {Cu(I)−Cu$_2$(I,II)−Cu(II)} species is formed at the first reduction. This might be taken as an indication that one auxiliary and the dimetallic core are reduced by chance at the same potential (ca. -0.08 V). However, it must be emphasized that the first reduction potential corresponds well to the reduction potential of the monometallic reference, and the reduction of the dimetallic reference occurs at a much lower potential (ca. -0.67 V). It is most likely that the two auxiliaries are reduced at ca. -0.08 V and intramolecular electron transfer occurs from one reduced auxiliary to the dimetallic core: {Cu(II)−Cu$_2$(II,II)−Cu(II)}/{Cu(I)−Cu$_2$(II,II)−Cu(I)} → {Cu(I)−Cu$_2$(I,II)−Cu(II)}. On the basis of this presumption, the second reduction wave at ca. -0.42 V involving one-electron transfer can be assigned to the process: {Cu(I)−Cu$_2$(I,II)−Cu(II)}/{Cu(I)−Cu$_2$(I,II)−Cu(I)}.

The CV of **2** in DMSO has two quasi-reversible or reversible couples at -0.68 and -0.99 V (vs SCE). Coulometric studies at -0.77 and -1.08 V demonstrated one-electron transfer, respectively. The electrolyzed solution at -0.77 V showed an axial pattern of EPR with $g_\parallel = 2.18$, $g_\perp = 2.06$, and $A_\parallel = 196 \times 10^{-4}$ cm^{-1} (in frozen DMSO solution) attributable to the monometallic auxiliary. A detailed inspection of the EPR spectrum revealed another EPR signal of a smaller hyperfine structure. For the two-electron reduced solution at -1.08 V, the second EPR signal was well resolved and assigned to the dimetallic core in the spin-trapped Cu$_2$(I,II) state. The CV of **2** can be interpreted in terms of the stepwise reductions: {Cu(II)−Cu$_2$(II,II)−Cu(II)}/{Cu(II)−Cu$_2$(I,II)−Cu(II)}/{Cu(I)−Cu$_2$(I,II)−Cu(II)}.

Chemical Reduction. Chemical reduction of **1** and **2** with ascorbic acid and sodium dithionite was examined by EPR spectroscopy at liquid nitrogen temperature. Assembly **1** was reduced with ascorbic acid, whereas the assembly **2** could not be reduced with ascorbic acid. In Figure 4 are shown EPR spectral changes on adding ascorbic acid to a DMSO solution of **1**. With [ascorbic acid]/[**1**] = 1/2 (in molar ratio), the EPR spectrum has both characteristics of the monometallic auxiliary and the spin-localized dimetallic Cu$_2$(I,II) core (trace a). When [ascorbic acid]/[**1**] = 3/2, the spectrum has only the signal of the spin-localized Cu$_2$(I,II) species (trace b). This spectrum b is essentially the same as spectrum b in Figure 3. Further addition of ascorbic acid gave no change in the EPR spectra. The result clearly demonstrate that assembly **1** is reduced to {Cu(I)−Cu$_2$(I,II)−Cu(I)} with ascorbic acid.

When sodium dithionite was used as the reducing agent, assembly **1** was fully reduced to {Cu(I)−Cu$_2$(I,I)−Cu(I)}, as judged from discoloration of the complex solution, and then resulted in the deposition of metallic copper. This is consistent with the further electrochemical reduction of {Cu(I)−Cu$_2$(I,II)−Cu(I)}, causing the deposition of metallic copper on the electrode.

Relevance to Multicopper Oxidases. On the basis of the above discussion, it is evident that assembly **1** is reduced to {Cu(I)−Cu$_2$(I,II)−Cu(I)} through an intramolecular electron transfer from one of the reduced auxiliaries to the dimetallic core. As the result, the dimetallic core in **1** is reduced at a potential significantly higher than that required for reducing the reference core. It must be emphasized that such an intramolecular electron transfer does not occur for a mixture of [Cu$_2$(fsac-bz)$_2$] and Cu(dpa)Cl$_2$ (1:2 molar ratio). Recently, we have observed a similar electron transfer for a related tetracopper

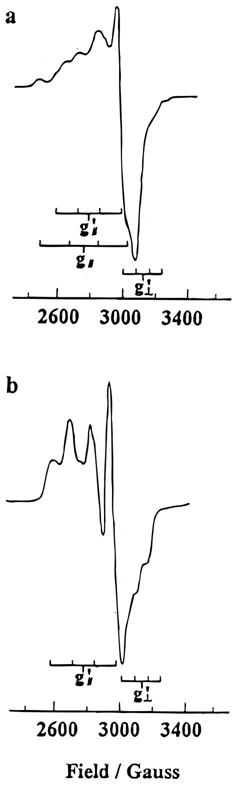
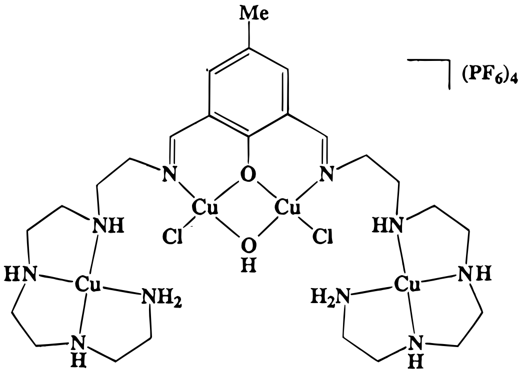


Figure 4. EPR spectral changes of **1** (in frozen DMSO) on adding ascorbic acid: (a) [ascorbic acid]/[**1**] = 1/2 (in molar ratio) and (b) [ascorbic acid]/[**1**] = 3/2.

assembly complex (Chart 4),[32] where a rare isomerization of the linear pentamine, incorporated in the Schiff-base framework, into a branched form was recognized after electrochemical reduction and reoxidation with dioxygen. It appears that an interaction between the dimetallic core and the reduced monometallic auxiliaries gives rise to a specific stabilization, and the amine isomerization occurs in the stabilized form. A similar interaction within a molecule to stabilize the dimetallic core must be the cause for the intramolecular electron transfer for **1**, and this can be also the case of biological multicopper oxidases.

Two mechanisms are proposed for the reduction of ascorbate oxidase based on kinetic studies.[33] In mechanism I, the reduction of type 1 Cu precedes the reduction of type 2 Cu, followed by intramolecular two-electron transfer from reduced type 1 and type 2 sites to a type 3 site to provide the fully reduced Cu$_2$(I,I) state for type 3. In mechanism II, the first reduction occurs at type 1 and an intramolecular electron transfer occurs from the reduced type 1 site to type 3 site to provide a mixed-valence Cu$_2$(I,II) state for type 3. The subsequent reduction at type 1 and the intramolecular electron transfer to type 2, followed by the reduction at type 1 and the electron transfer to type 3, forms the fully reduced type 3 center. Thus, the intramolecular electron transfer observed for **1** is relevant to the initial stage of mechanism II supposed for ascorbate oxidase. There are substantial differences in behavior between **1** and biological multicopper oxidases, especially: (1) the dimetallic core of **1** is hardly reduced to Cu$_2$(I,I) and (2) the resulting Cu$_2$(I,I) species is unstable. The type 3 site in multicopper oxidases exists as triangular (type 2)/(type 3) cluster that must be important for stabilizing the fully reduced Cu$_2$(I,I) state for type 3. Another point to be mentioned is that the dimetallic core of **1** is held in a rigidly coplanar mode and has little flexibility for geometric change, whereas type 3 copper is considered to have a flexible dinuclear core as judged from its high reduction potential. Such flexibility of the dimetallic core must be important to achieve the fully reduced Cu$_2$(I,I) state of type 3.

Chart 4. Chemical Structure of a Related Assembly Complex



In conclusion, assembly **1** differs from multicopper oxidases in the arrangement of the copper centers but shows an intramolecular electron transfer relevant to multicopper oxidases.

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