CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UTAH STATE UNIVERSITY, LOGAN, UTAH,
AND THE INSTITUTE OF INORGANIC CHEMISTRY, UNIVERSITY OF BASEL, BASEL, SWITZERLAND

A Binuclear Mixed-Valence Copper Acetate Complex as a Model for Copper–Copper Interaction in Enzymes[1]

BY C. SIGWART, P. HEMMERICH, AND J. T. SPENCE

A Cu(I)–Cu(II) mixed-valence acetate complex has been prepared in methanol. The complex is deep violet, with absorption maxima at 508 and 900 mμ. Continuous variation studies showed the Cu(I):Cu(II) and the copper:acetate ratios of the complex to be 1:1. Esr measurements in methanol gave a seven-line spectrum, indicating the two copper ions are indistinguishable and their formal oxidation states indeterminate. The complex was also prepared using ^{13}C acetate labeled in the carboxy group, but the esr spectrum of this species did not show any additional splittings due to the ^{13}C nucleus. A possible structure and the implications for copper enzymes are discussed.

The nature of the bonding of copper, as well as its valence state, is still a matter of controversy for many of the copper enzymes. A number of these enzymes contain more than one copper per molecule and, in some cases, the copper cannot all be accounted for by esr as Cu(II) in the oxidized enzyme ($\sim$40% in cytochrom c oxidase, e.g.).[2] To explain this and the unusual absorption and esr spectra of these enzymes, an interaction between copper ions in close proximity was originally suggested by Blumberg *et al.*,[3] Malmstrom,[4] and Beinert, *et al.*,[2] although no evidence to support this suggestion was obtained in the case of cytochrome oxidase.[5] More recently, with the finding of similar spectral properties in proteins having only a single atom of copper per molecule, such as stellocyanin,[6] copper–copper interaction has seemed much less likely and the spectra have been attributed to Cu(II) in highly distorted ligand fields. The results reported here indicate that such absorption spectra, not commonly found in model Cu(II) complexes, may also arise as a result of Cu(I)–Cu(II) interaction in mixed-valence complexes.

Data from model Cu(I)–Cu(II) mixed-valence complexes have been difficult to obtain, owing mainly to the problem of finding ligands which do not greatly favor one oxidation state and to the sensitivity of such mixed-valence systems to oxygen. Until now, only a few copper mixed valence species have been investigated and these cannot be regarded as good models for enzymes. Furthermore, most of these systems are, according to the classification system of Robin and Day,[7] in class I (metal ions in ligand fields of very different geometry, showing no mixed-valence absorption in the visible region and clearly showing the spectra of the constituent ions). These compounds cannot explain the deep color of copper proteins nor the special redox ability of the copper enzymes. For these enzymatic redox processes, a binding site for copper which does not change ligand geometry drastically during the uptake or loss of an electron is needed. A mixed-valence complex with rapid electron exchange would meet this criterion. Such a complex should have the characteristics summarized by Robin and Day in their description of class IIIa mixed-valence compounds:[7] metal ions indistinguishable but grouped into polynuclear clusters with one or more mixed-valence transitions in the visible region and spectra of the constituent ions not discernible.

Because of the low copper content in copper proteins, the active site must be a small unit with one to eight copper ions. Therefore, the species [Cu$^I_2$(RS)$_2\leftrightarrow$(Cu^{II}SR)$_2$]$^{2+}$, first reported by Klotz, *et al.*,[8] and reinvestigated by Hemmerich, *et al.*,[9] may be regarded as a reasonable approximation to natural systems. Since the structure of this compound and the influence of the valence state of the "noninnocent ligands"[10] is still a matter of controversy, however, little can be said about the interaction of the copper ions.

The best investigated mixed-valence copper complex is the Cu(I)–Cu(II) chloride system. McConnell and Davidson[11] found this complex in chloride solution to have the stoichiometric formula Cu$_2$Cl$_3$ and to exhibit new absorption in the visible region. The solid hexaamminecobalt(III) chlorocuprates(I,II) show similar spectra in the visible region, the absorption being due to the presence of both valence states (580 mμ for the solid compound and 555 mμ for the system in solution). Day and Smith[12] assigned the absorption in the case of this solid to an intermolecular charge transfer from a chlorocuprate(I) anion to a chlorocuprate(II) anion. From the similarity of the absorp-

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(4) B. G. Malmstrom, "Oxidases and Related Redox Systems," T. E. King, H. S. Mason, and M. Morrison, Ed., John Wiley & Sons, Inc., New York, N. Y., 1965, p 207.

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(7) M. B. Robin and P. Day, *Advan. Inorg. Chem. Radiochem.*, **10**, 247 (1967).

(8) I. M. Klotz, G. H. Czerlinski, and H. A. Fiess, *J. Am. Chem. Soc.*, **80**, 2920 (1958).

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(10) C. K. Jørgenson, *Struct. Bonding* (Berlin), 1, 234 (1967).

(11) H. M. McConnell and N. Davidson, *J. Am. Chem. Soc.*, **72**, 3168 (1950).

(12) P. Day and D. W. Smith, *J. Chem. Soc., A*, 1045 (1967).

tion spectra, Robin and Day[7] concluded that the same assignment can be made for this mixed-valence system in solution.

In the course of an investigation of Cu(I) complexes, it was discovered that a deep violet color was formed upon the addition of $Cu(CH_3CN)_4ClO_4$ to a solution of $Cu(ClO_4)_2 \cdot 6H_2O$ in acetate-buffered methanol. Because of the similarity of the absorption spectra to that of some copper enzymes, an investigation of this complex as a possible model for copper–copper interaction in these enzymes was undertaken.

Results

The absorption spectrum of the complex in the visible region shows a sharp peak at 508 mμ and a broad peak centered at 900 mμ, indicative of interaction between the two valence states of copper,[7] since these peaks do not exist in either of the component species (Figure 1). The composition of the mixed-
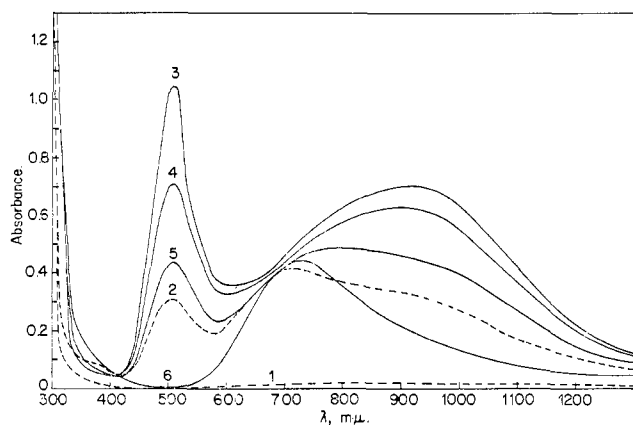
Figure 1.—Spectrophotometric characterization of the binuclear mixed-valence copper acetate complex. The ratio Cu(II):Cu(I) increases from curve 1 to curve 6: – – – –, Cu(II):Cu(I) < 1; ———, Cu(II):Cu(I) ≥ 1. Curve 1: 2.00×10^{-2} M $Cu^I(CH_3CN)_4ClO_4$ and 1.00×10^{-1} M CH_3COOH in absolute methanol. Curve 2: 4.00×10^{-3} M $Cu^I(CH_3CN)_4$, 1.00×10^{-3} M $Cu^{II}(ClO_4)_2 \cdot 6H_2O$, 5.00×10^{-3} M CH_3COONa, and 1.00×10^{-1} M CH_3COOH. Curve 3: 2.50×10^{-3} M $Cu^I(CH_3CN)_4$-ClO_4, $2.50 \times 10^{-3}M$ $Cu^{II}(ClO_4)_2 \cdot 6H_2O$, 5.00×10^{-3} M CH_3CO-ONa, and 1.00×10^{-1} M CH_3COOH. Curves 4–6 represent the slow autoxidation of the solution described under curve 3. Curve 6: completely oxidized.

valence compound was found by means of a continuous variation plot of the different species in dilute solution under anaerobic conditions. Figure 2 indicates that the absorption at 508 mμ is a maximum with a Cu(I): Cu(II) ratio of 1:1 (ϵ 416). The absorption also reaches a maximum when the Cu(total):acetate ratio is 1:1. With a higher ratio of acetate, a colorless solid precipitated from the solution.

No spectral evidence for a mixed-valence complex was observed with unsaturated or saturated dicarboxylic acids, pyridine, urea, or thiourea. With benzoate, however, a similar mixed-valence complex was formed (ϵ_{508} 358).

The absorption was found to be dependent on the solvent. The highest absorption was observed in
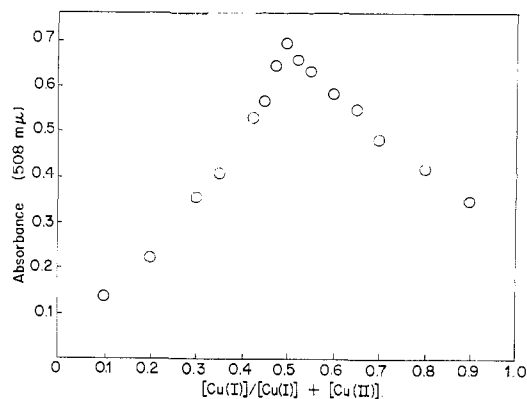
Figure 2.—Continuous-variations plot for the determination of the Cu(I):Cu(II) ratio of the mixed-valence complex. Absorption at 508 mμ is plotted *vs.* the fraction of Cu(I). [Cu(I)] + [Cu(II)] = 5.00×10^{-3} M, [CH$_3$CN] = 2.00×10^{-2} M, [CH$_3$COONa] = 5.00×10^{-3} M, and [CH$_3$COOH] = 1.00×10^{-1} M, in absolute methanol.

methanol. In the series of saturated alcohols the absorption diminishes with increased chain length and decreasing dielectric constant. Other solvents, such as tetrahydrofuran or dioxane, in which the Cu(I) and Cu(II) salts were soluble, gave only very weak absorption. The mixed-valence complex is apparently not formed in water.

Additional acetonitrile suppressed the formation of the mixed-valence complex, in accordance with the results of Manahan and Iwamoto.[13] Addition of a small amount of water to the complex caused no change of absorbance in the range of 0–30 mol of H_2O/mol of Cu(II), while more water destroyed the complex.

In order to obtain more information about the structure of the mixed-valence complex, esr measurements were made at room temperature and at $-195°$. At room temperature, the complex gives an esr spectrum with seven hyperfine lines, with $g = 2.17$ and $A = 42$ G (Figure 3), indicating that the two coppers are not distinguishable by esr and that the electron is equally delocalized on both coppers. This appears to be the first reported esr spectrum of a mixed-valence Cu(I)–Cu(II) complex showing such delocalization. The esr spectrum for a somewhat similar binuclear cobalt complex, $[(NH_3)_5CoOOCo(NH_3)_5](NO_3)_5$, shows 15 lines and has also been interpreted in terms of equal delocalization of the electron on both cobalt nuclei.[14] The interpretation of the low-temperature spectrum (Figures 4 and 5) is difficult, owing to the large number of lines. The low-temperature esr spectrum probably represents contributions from both the mixed-valence complex and a Cu(II) species, since the complex tends to dissociate at temperatures below $\sim-50°$ into its components, even with rapid freezing in liquid nitrogen. Repeated attempts to obtain a better spectrum failed, even when the solution was injected from a syringe directly into liquid nitrogen in the dewar. The line at $g = 2.37$ (Figure 5) is most likely the center of the anticipated seven-line $g_{\parallel}$ of the mixed-valence

(13) S. E. Manahan and R. T. Iwamoto, *Inorg. Chem.*, **4**, 1409 (1965).
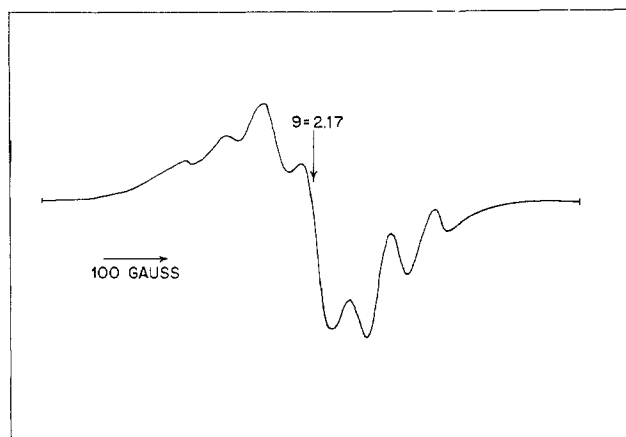(14) E. A. V. Ebsworth and J. A. Weil, *Proc. Chem. Soc.*, 57 (1959).

Figure 3.—Room-temperature esr spectrum of the mixed-valence complex. $[Cu(I)] = 5.00 \times 10^{-3} M$, $[Cu(II)] = 5.00 \times 10^{-3} M$, $[CH_3COONa] = 1.00 \times 10^{-2} M$, and $[CH_3COOH] = 1.00 \times 10^{-1} M$.
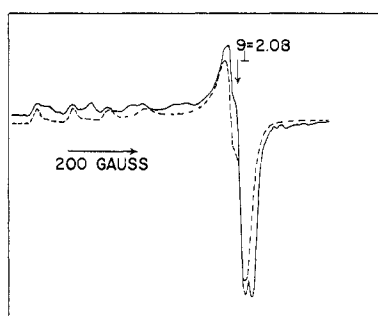

Figure 4.—Esr spectra at $-195°$: ———, mixed-valence complex, $[Cu(I) = 5.00 \times 10^{-3} M$, $[Cu(II)] = 5.00 \times 10^{-3} M$, $[CH_3COONa] = 1.00 \times 10^{-2} M$, $[CH_3COOH] = 1.00 \times 10^{-1} M$; – – – –, same, but without Cu(I).
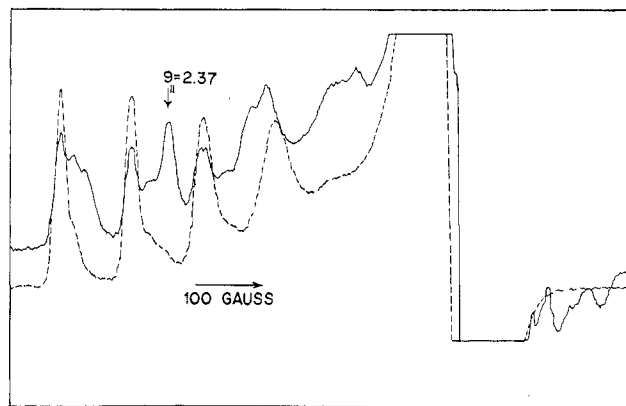

Figure 5.—This is the same as Figure 4 but at 12.8 times increased signal level to show hyperfine splittings.

spectrum. In fact, using the expression $g = \frac{1}{3}(g_{||} + 2g_{\perp})$, a value of 2.18 is obtained, in good agreement with 2.17 for the measured room-temperature value.

Discussion

The results lead to the following conclusions concerning the mixed-valence complex.

(1) Strong Cu(I) or Cu(II) ligands (H₂O, pyridine, etc.) interfere with the formation of the mixed-valence complex.

(2) As indicated by the continuous variation curve, the mixed-valence complex is rather strong, certainly stronger than either of its component complexes.

(3) The two copper ions are in the same ligand field and are equivalent, either because the electron oscillates between both nuclei faster than the resolving time of the esr equipment or because the odd electron is found in a molecular orbital encompassing both copper ions. In regard to this, the rate constant for the electron exchange reaction $Cu^{2+} + Cu^+ \rightleftarrows Cu^+ + Cu^{2+}$ was measured by nmr line broadening and found to be $5 \times 10^7 M^{-1} sec^{-1}$ in 10 N HCl.[15] On the basis of the esr hyperfine splitting (42 G), the minimum exchange rate for the mixed-valence complex was estimated to be $1.3 \times 10^8 sec^{-1}$.

In order to obtain additional information concerning interaction between the copper ions, measurements of the esr line widths were made at different temperatures between -50 and $+50°$. The observed differences, however, were of the same magnitude as the error (± 1 G). As indicated above, the mixed-valence complex begins to dissociate into its components below $-50°$. This dissociation was shown by the reversible change in both visible and esr spectra when the temperature was slowly lowered. At temperatures $< -50°$ the visible spectrum becomes essentially identical with that obtained upon oxidation of the mixed-valence complex to Cu(II), and the seven-line esr spectrum changes to that of Cu(II). Both spectra return to that of the original complex upon warming again.

Finally, the complex was prepared using ^{13}C-labeled acetate (labeled in the carboxy carbon) in order to determine if additional splittings of the seven-line esr spectrum due to the ^{13}C nucleus ($I = \frac{1}{2}$) could be observed. The spectrum was identical, however, with that of the natural isotope species. This might be due to very small splittings or to no appreciable delocalization of the electron on the carboxylate carbon. Thus the mechanism of electron exchange is not clear, although the constancy of the esr line widths over the temperature range studied favors a tunneling mechanism.

These results suggest as a possible structure a binuclear complex with oxygen bridges, similar to the structure proposed by Hemmerich, *et al.*, for the CuS_2Cu complex.[9]
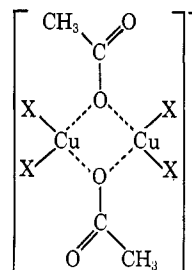
In view of the dependence of complex formation on solvent, the other coordinating groups (X) are most likely CH_3OH and the geometry could be tetrahedral,

(15) H. M. McConnell and H. E. Weaver, *J. Chem. Phys.*, **25**, 307 (1956).

square planar, or an intermediate distorted arrangement. At present, this structure must be regarded as tentative, although the negative results with the ^{13}C acetate make it more likely than a structure involving bridging carboxylate groups, as has been determined by X-ray crystallography for $Cu_2(CH_3-COO)_4 \cdot 2H_2O$.[16]

These results indicate the unusual absorption spectra observed with some of the copper enzymes could be due to Cu(I)–Cu(II) interaction, as well as be caused by Cu(II) in a highly distorted ligand field. In cytochrome c oxidase, *e.g.*, a broad band, centered at 830 mμ, is attributed to the copper chromophore.[17] The broad band with center at 900 mμ of the mixed-valence model complex could well correspond to this feature of cytochrome c oxidase.

No seven-line esr spectra of copper enzymes have been published. This does not exclude enzymatic copper–copper interaction, however, since if the exchange rate in the enzyme is considerably lower than in this model, a four-line spectrum would be observed.

Experimental Section

The spectrometric measurements were carried out with a Cary 14 E spectrophotometer. In order to exclude oxygen, a 1-cm cuvette with two side arms and a stopcock that could be flushed with N_2 and sealed off was used. The absolute methanol with suitable amounts of acetic acid and sodium acetate was deaerated in the cuvette with prepurified N_2 for 15 min and the solid copper(I) and copper(II) compounds were then introduced into the

side arms. The solution and the cuvette were again deaerated for 15 min and then shaken for 5 min to mix the contents. The absorption was reproducible within $\pm 2\%$ absorbance. The color is stable under complete exclusion of O_2 but quickly changes to the blue of Cu(II) in the presence of traces of O_2.

Room-temperature esr measurements were made with a Varian V-4500-10, X-band spectrometer equipped with a variable-temperature accessory, using 100-kc modulation, in a flat quartz cell that was sealed after filling under an atmosphere of N_2. The g value was determined by comparison with the signal of 2,2-diphenyl-1-picrylhydrazyl in benzene. The low-temperature spectra were obtained by rapidly freezing the solution of the complex in liquid nitrogen. In order to obtain the complex under these conditions, it was found necessary to bubble He through the solution for at least 20 hr before freezing; otherwise, the complex immediately dissociated into green Cu(II) upon freezing. This is most likely due to the presence of a small amount of CH_3CN in the solution, which favors the formation of a Cu(I) complex over the mixed-valence complex at low temperatures (see results above). CH_3CN is probably removed by long bubbling with He, preventing the preferential formation of this complex upon freezing.

Materials. $Cu(ClO_4)_2 \cdot 6H_2O$, purchased from Fluka or G. Frederich Smith Co., was used without further purification; $Cu(CH_3CN)_4ClO_4$ was prepared as described previously;[18] absolute methanol, distilled from $Mg(OCH_3)_2$, and acetic acid (Fluka p.A.), distilled over chromic acid, were used. Sodium acetate (p.A.), free of water, was obtained from Siegfried Co. The ^{13}C sodium acetate (60% enrichment) was purchased from Nuclear Research Chemicals and used without purification.

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(17) H. Beinert, "The Biochemistry of Copper," J. Peisach, P. Aisen, and W. E. Blumberg, Ed., Academic Press, New York, N. Y., 1966, p 213.

(18) P. Hemmerich and C. Sigwart, *Experientia*, **19**, 488 (1963).

Single-Crystal Electron Paramagnetic Resonance Studies of Copper Diethyldithiocarbamate

BY M. J. WEEKS AND J. P. FACKLER

Single-crystal electron paramagnetic resonance studies have been carried out on copper(II) diethyldithiocarbamate doped into both the zinc and nickel diethyldithiocarbamate complexes. The crystal structures of all three complexes are known, and the orientation of the principal axes of the g and hyperfine tensors can be related to the molecular axes. The lowering of symmetry on going from the nickel to the zinc diethyldithiocarbamate host leads to significant changes in the isotropic as opposed to the anisotropic hyperfine interaction. These changes have been attributed to increased orbital mixing upon a lowering of symmetry about the metal ion. The epr results were used to obtain molecular orbital coefficients from standard expressions employing orbitals based on the metal ion and sulfur atoms. The coefficient values were tested for their sensitivity to the various parameters in the expressions, in particular, ligand spin–orbit coupling. Molecular orbital coefficients obtained were compared with those from a Wolfsberg–Helmholtz calculation.

Introduction

In recent years there had been interest, both in this laboratory[1] and elsewhere,[2] in the complexes formed by

(1) J. P. Fackler, Jr., D. Coucouvanis, J. A. Fetchin, and W. C. Seidel, *J. Am. Chem. Soc.*, **90**, 2784 (1968).

(2) H. B. Gray, *Progr. Transition Metal Chem.*, **1**, 239 (1965).

transition metals with chelating ligands bonding through sulfur. In this paper, we report the results that have been obtained from single-crystal epr studies of copper(II) diethyldithiocarbamate, $Cu(dtc)_2$, doped into single crystals of both the zinc and nickel diethyldithiocarbamate complexes. Pettersson and