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Transient Intermediates in the Copper-catalyzed Oxidation of Cysteine

Recently, the formation of short-lived intermediate in many metal ion-catalyzed redox reactions has been proved spectrophotometrically.[1] In the copper ion-catalyzed oxidation of sulfhydryl compound, production of a transient purple color has been observed upon mixing the solutions containing Cu(II) and the substrate. A purple color observed in the interaction of Cu(II) with mercatosuccinic acid[2] or with penicillamine,[3] which is lasted for few seconds, is attributed to a mixed-valence complex of copper. The chemical structure of the purple species in the solution has been uncertain. On the other hand, since a purple color in the Cu(II)-cysteine interaction is fleetingly faded, precise description about the structure for the purple species has hitherto been defied. We have employed a continuous-flow technique to obtain direct evidence for the existence of transient species with half-lives of formation and decomposition of the order of milliseconds. In the present paper was communicated some properties of the purple species produced in the Cu(II)-cysteine interaction, which was examined with the electronic absorption and electron spin resonance (ESR) spectroscopies.

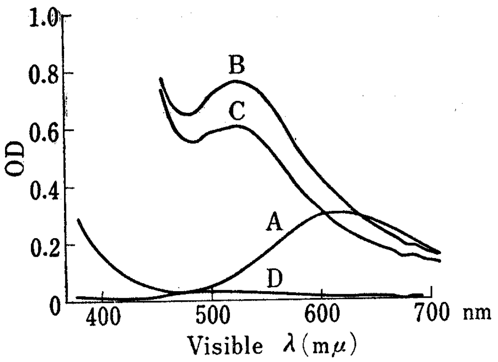

Fig. 1. Oscillograph Traces of the Electronic Absorption Spectra of the Purple Species Produced in the Cu(II)-Cysteine Interaction at pH 7.5 and 20° under Nitrogen

[Cu(II)] $= 3.5 \times 10^{-3}$M; [glycylglycine] $= 3.5 \times 10^{-2}$M; [cysteine] $= 3.5 \times 10^{-2}$M; ionic strength $= 0.25$ (KNO_3) time after mixing: A, 0 msec (Cu(II)-glycylglycine); B, 6 msec; C, 22 msec; D, 1 sec
The measurement was done with a Hitachi RSP-2 rapid scan spectrophotometer in 1.0 cm cell.

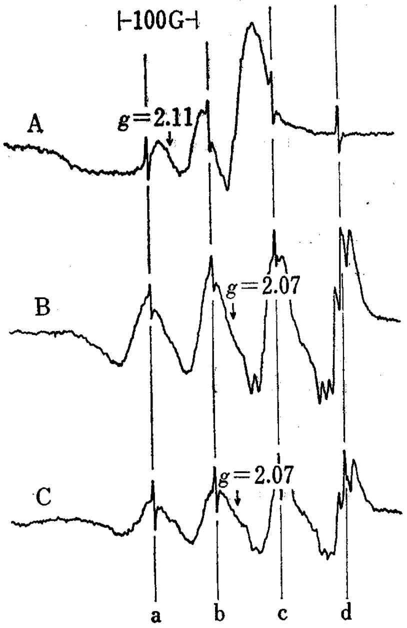

Fig. 2. ESR Spectra of the Purple Species at pH 7.5 and at Room Temperature in an Air-saturated Solution

[Cu(II)] $= 3.5 \times 10^{-3}$M; [glycylglycine] $= 3.5 \times 10^{-2}$M; [cysteine] $= 3.0 \times 10^{-2}$M; ionic strength $= 0.25$(KNO_3) time after mixing: A, 0 msec (Cu(II)-glycylglycine); B, 9 msec; C, 51 msec
ESR spectroscopy was performed with a JEOL JES-MEX band spectrometer equipped with a 100 KHz field modulation unit. a, b, c and d indicate the signals of Mn^{2+} as a field marker.

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When an aqueous solution of cysteine is mixed within a few msec. with Cu(II)-glycylglycine, a change in the electronic spectrum occurs which is not attributed to either the reactants or the final oxidation products. The electronic spectra at various times after mixing are shown in Fig. 1. Formation of the purple species was extremely rapid so that considerable color change was observed within the dead time of the instrument. Under the experimental condition described under Fig. 1, the optical density at $\lambda_{max}=530$ nm, abbreviated as OD_{530}, reached a maximum within 10 msec. after mixing and diminished thereafter. The time course of OD_{530} shows the formation and subsequent decomposition of the purple intermediate.

When the Cu(II)-cysteine interaction is monitored with the ESR spectroscopy, a change in the spectrum is observed. The ESR spectra during the reaction at room temperature is demonstrated in Fig. 2. The spectrum at the first, where Cu(II) is complexed with glycylglycine, consists of four hyperfine lines, due to Cu(II), centered at an average g value, $g_{av}=2.115\pm0.005$, with an average hyperfine splitting, $A_{av}=(54\pm1)\times10^{-4}\ \mathrm{cm}^{-1}$. This spectrum is deformed instantaneously upon mixing Cu(II)-glycylglycine and cysteine. The spectrum of the purple species consists of four copper hyperfine lines with $g_{av}=2.065\pm0.005$ and $A_{av}=(82\pm1)\times10^{-4}\ \mathrm{cm}^{-1}$ and shows an additional ligand hyperfine splitting of at least five lines, which is varied depending on the ratio of cysteine to Cu(II). This indicates that Cu(II) is complexed to at least two nitrogen atoms; at least one of which is derived from cysteine. The ESR signal was diminished at a corresponding rate to the decrease of OD_{530} upon stopping flow of the sample solution. When the purple color was faded away, the ESR signal was disappeared completely. Those finding obtained from the electronic and ESR spectroscopies indicate clearly that Cu(II) associates with cysteine to form short-lived paramagnetic Cu(II) complexes which subsequently undergo the intramolecular electron transfer to yield colorless Cu(I) complexes.

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