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AN ELECTRON SPIN RESONANCE STUDY OF THE OXIDO-REDUCTIVE INTERACTION OF Cu(II)-GLYCYLGLYCINE AND CYSTEINE IN A NEUTRAL AQUEOUS SOLUTION

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The oxido-reductive interaction of Cu(II)-glycylglycine and cysteine was examined with electron spin resonance (ESR) spectroscopy using a continuous-flow technique. Firstly, Cu(II)-glycylglycine associates with cysteine to form a ternary complex, which is decomposed subsequently (relaxation time = 150 msec) to ESR silent species. When an excess of cysteine is added, the ternary complex is transformed to unidentified Cu(II) species, probably Cu(II)-cysteine complexes, undergoing rapid decomposition to Cu(I) species.

Production of a transient purple color upon mixing solutions of Cu(II) and sulfur-containing compounds in a neutral or slightly alkaline medium has been observed in many laboratories. Earlier workers studied the formation of the purple species in a state of equilibrium and assumed that the purple color is due to a mixed-valence complex of copper.[1] However, because of the extreme lability of the purple species and the complexity of the reaction system containing multi-species, the lack of the reproducibility of the results is commented. We reported in a previous paper that the purple color is due to intermediates which appears at an earlier stage of the oxidation of the sulfur-containing compound by Cu(II).[2] Since the purple color appears transiently, measurement should be done using a rapid-scanning or continuous-flow technique. In the present communication, an electron spin resonance (ESR) study of the transient species with half-lives of the order of milliseconds is described.

Copper(II) chloride, glycylglycine and cysteine were obtained from commercial sources of reagent grade and used without further purification. Water was triply distilled, the second distillation being from alkaline permanganate. A stock solution of Cu(II) prepared was standardized against EDTA with the complexometric titration.[3] A Cu(II) solution (solution A) contains

6.0×10^{-3} M Cu(II) and tenfold excess of glycylglycine over Cu(II). Another solution (solution B) contains various amounts of cysteine. Both solutions were prepared just prior to use.

The formation and subsequent decomposition of the purple species was observed in an air-saturated solution.[4] Solutions A and B were mixed with a JES-SM-1 rapid mixing device and the outlet of the mixing chamber was connected directly with a quartz observation tube (diameter = 0.95 mm). Dead volume of the instrument was either 0.037 ml or 0.092 ml, and dead time was controlled by varying the rate of delivery of the solution from the mixing chamber. ESR spectra were obtained on a JEOL JES-PE-3X spectrometer with 100 kHz modulation and a continuous-flow technique was employed. The values of g factor and hyperfine constant of the spectrum were calculated by comparison with a standard sample of Mn(II) in MgO. The condition of the ESR measurement was as follows; microwave power = 12.8 mW and modulation amplitude = 5 G.

The purple species with λ_{max} = 530 nm gives an ESR spectrum consisting of four hyperfine lines with an additional ligand hyperfine splitting of at least seven lines, which indicates that the complex contains paramagnetic copper coordinated with more than three nitrogen atoms derived from cysteine and/or glycylglycine. Since the formation of the complex is very rapid undergoing almost completely within the dead time of the instrument, the decomposition process was examined with ESR spectroscopy.

ESR parameters; i.e., g and A values, of the complex vary depending upon the ratio of the concentration of cysteine to Cu(II), which indicates that the purple complex does not correspond to a single molecular species. At [cysteine]/[Cu(II)] = 1, the ESR parameters do not vary and show constant values ($g = 2.008 \pm 0.001$, $A = (77 \pm 1) \times 10^{-4}$ cm^{-1} and ligand hyperfine splitting = 7 lines) during the reaction. This suggests that the complex is not modified structurally in the course of the reaction. An ESR titration data presents a possibility of the formation of a ternary complex composing of Cu(II), glycylglycine and cysteine.[5] The ternary complex is decomposed rapidly, showing a single relaxation curve (relaxation time = 150 msec), to yield ESR silent species. The ESR signal is thus disappeared within one second.

When an excess of cysteine over Cu(II) is added to the reaction medium, the ESR parameters, as well as the signal height, vary depending upon the ratio of [cysteine] to [Cu(II)] and the reaction time. At [cysteine]/[Cu(II)] = 4, the spectrum at an earlier stage, resembling that of the ternary complex, rather displays a complicated pattern. As the decomposition reaction proceeds, the spectrum becomes more complicated. The ESR signal observed may be resolved into two spectra.

One is due to the ternary complex which decreases first. Another is due to unidentified species which may be originated from the ternary complex and is decomposed later. Probably, free cysteine associates with the ternary complex, substituting with glycylglycine molecule to form Cu(II)-cysteine binary complexes. Under those conditions, the ternary complex is either decomposed to ESR silent species, or transformed to the binary complexes. Thus, at least two intermediates are involved in the Cu(II)-catalyzed oxidation of cysteine.

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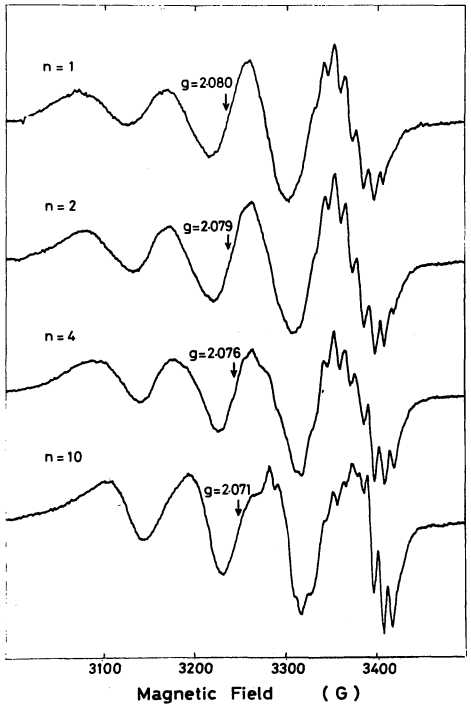

Fig. 1 ESR spectra of the purple species resulting from the interaction of Cu(II)-glycylglycine and cysteine : Effect of n = [cysteine]/[Cu(II)]
[Cu(II)] = 3.0×10^{-3} M, [glycylglycine] = 3.0×10^{-2} M
ionic strength = 0.5 (KCl), pH 7.5, room temperature

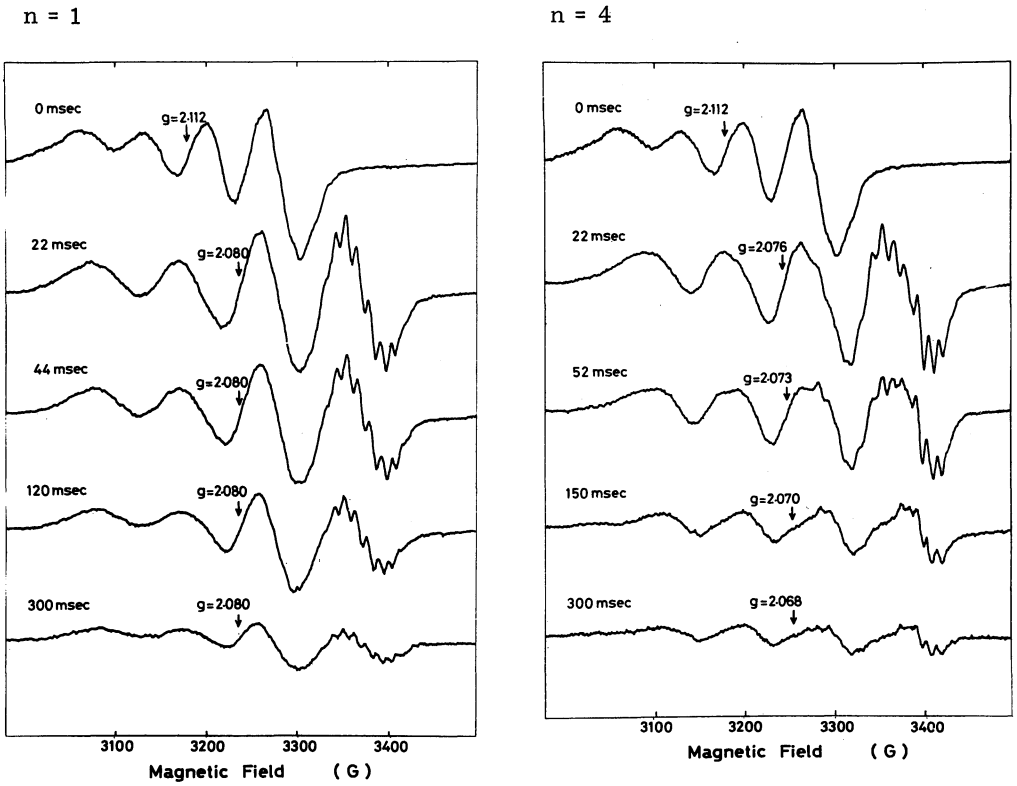

Fig. 2 Variation of the ESR spectra during the decomposition of the purple species

Experimental details as under Fig. 1

References and Notes

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4) Effects of oxygen dissolved in the medium were not observed at an earlier stage of the reaction.

5) The plot of the signal height against [cysteine]/[Cu(II)] shows an inflection point near n = 1, indicating the formation of a ternary complex. A detailed accout of the result will be presented in near future.

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