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Magnetic and EPR Studies on the Interaction of Copper(II)–Glycylglycine with Ferricyanide

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Introduction

Galactose oxidase, a copper enzyme, interacts with ferricyanide and this interaction is responsible for the disappearance of the EPR signal of Cu(II) in the enzyme [1–3]. Cleveland and coworkers [2] have suggested that the ferricyanide binds to the Cu(II) of the enzyme and is responsible for elimination of the EPR signal of Cu(II). Recently Pretty and coworkers [4] have suggested that the CN^- is possibly bridging between Fe^{III} and Cu^{II} in the oxidized cytohrome a_3 (Fe^{III}–CN–Cu^{II}). In view of the importance of complexes having cyanide bridge between Fe^{II} and Cu^{II} in the biological systems, we report here the results of interaction of Cu(II)-glygly and ferricyanide using magnetic susceptibility and EPR methods. These results have led us to suggest a complex between Cu(II)-glygly and ferricyanide with a cyanide bridge.

Experimental

The Cu(II)-glycylglycine (Cu(II)-glygly) was prepared by the method of Martell and coworkers [5]. The potassium cobalticyanide, $K_3[Co(CN)_6]$, was prepared by a standard method [6]. The reaction product was twice recrystallized from water. The analytical reagent grade potassium ferricyanide, $K_3[Fe(CN)_6]$, was used. Other chemicals used were of laboratory reagent grade.

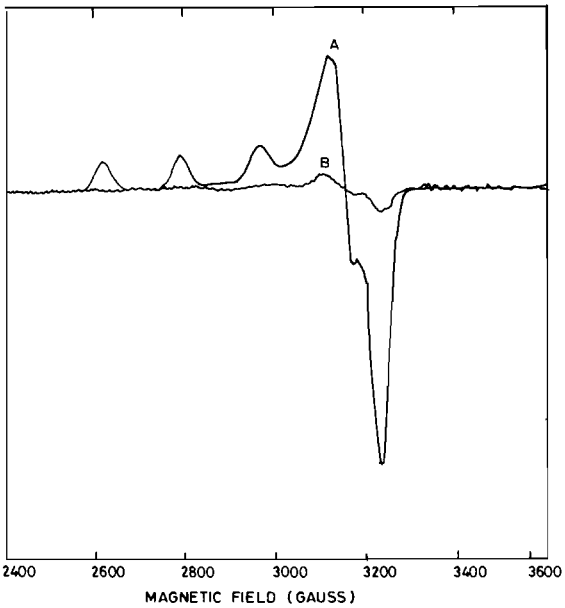

Fig. 1. EPR spectra of 0.9 m*M* copper(II)-glycylglycine + x m*M* ferricyanide. Curve A: x = 0. Curve B: x = 1.0 m*M*.

The magnetic susceptibility measurements [7] in solution were carried out by the Gouy method using a thin pyrex glass tube (1.2 cm internal diameter and 15 cm long). The calibration of the balance was done by using conductivity water and this water was also used as reference solvent. All susceptibility measurements were carried out at room temperature.

The electron paramagnetic resonance (EPR) measurements were made on a Varian E-4 spectro-

TABLE I. Magnetic Susceptibility Measurements of Cu(II)-glygly Complexes in Solution at 300 K.

Complex	$\chi_M^{cor} \times 10^6$ [a]	μ_{eff} (B.M.)
1. Cu(II)-glygly·$3H_2O$	1535	1.92
2. $K_3[Fe(CN)_6]$	2515	2.46 [7]
3. Cu(II)-glygly·$3H_2O$ + $K_3[Fe(CN)_6]$ (molar ratio is 1:1)[b]	2122 (4050)[c]	2.26 (3.12)
4. Cu(II)-glygly·$3H_2O$ + $K_3[Fe(CN)_6]$ + $K_3[Co(CN)_6]$ (molar ratios are 1:1:8)	3887	3.05

[a]Corrected molar susceptibility. [b]The Cu(II)-glygly and $K_3[Fe(CN)_6]$ in molar ratio of 1:1.2 was, in fact, used and the molar susceptibility of excess 0.2 *M* $K_3[Fe(CN)_6]$ was corrected. [c]The sum of corrected molar susceptibility values of Cu(II)-glygly and $K_3[Fe(CN)_6]$ in molar ratio of 1:1.

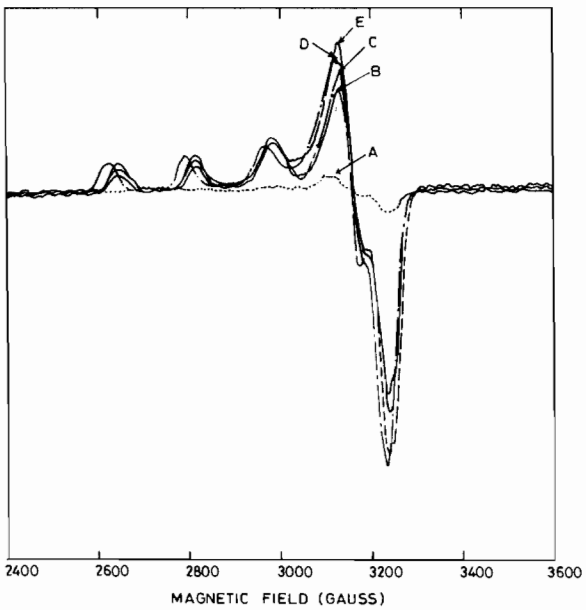

Fig. 2. EPR spectra of 0.9 m*M* copper(II)-glycylglycine + 1 m*M* ferricyanide + x m*M* cobalticyanide. Curve A: x = 0, curve B: x = 5.4 m*M*, curve C: x = 12.6 m*M*, curve D: x = 19.8 m*M*, and curve E: x = 27.0 m*M*.

meter (X-band) at liquid nitrogen temperature. All samples used for EPR studies were in 50% ethylene glycol and they were incubated for one hour at 20 °C before cooling to liquid nitrogen.

Results and Discussion

The EPR spectrum of Cu(II)-glygly at liquid nitrogen [8] is shown in Fig. 1, curve A ($g_{\parallel}$ = 2.262 and $g_{\perp}$ = 2.034). The EPR signal of this Cu(II) complex disappears in presence of about one mol of ferricyanide at liquid nitrogen temperature as shown in Fig. 1, curve B. The molar susceptibility of Cu(II)-glygly at room temperature in presence of one mol of ferricyanide is reduced to about half the value of the sum of the molar susceptibility values of Cu(II)-glygly and ferricyanide as shown in Table I. The areas of EPR peaks of Cu(II)-glygly in presence of one mol of ferricyanide at room temperature are also reduced to about half the value of the areas of EPR peaks of Cu(II)-glygly.

The interpretation of the above observations has been criticically discussed in terms of three possible mechanisms. In the first mechanism there is the possitility of charge transfer between Cu(II) and ferricyanide in the Cu(II)-glygly·ferricyanide complex. If this is the right mechanism, there must be a charge transfer band in the 200 to 400 nm region. No such charge transfer band is observed from 200 to 700 nm in the above complex. In this complex there is a visible band at 625 nm which is 25 nm shifted from the comparable intense 650 nm band of Cu(II)-glygly.

The disappearance of the EPR signal of Cu(II) in the Cu(II)-glygly·ferricyanide complex gives information about the ground state of the complex and therefore the charge transfer due to excited state of the complex is not responsible for elimination of the EPR signal of Cu(II). The absence of charge transfer band in the complex further rules out the first mechanism. In the second mechanism there is a possibility of nearly complete oxidation of Cu(II) to Cu(III) by adding one mol of ferricyanide. In this mechanism the diamagnetic Cu(III) may be responsible for the disappearance of the EPR signal at liquid nitrogen. The molar susceptibility of Cu(II)-glygly and ferricyanide in the molar ratio of 1:1 is expected to be around zero for this mechanism. Indeed, this mixture shows a molar susceptibility of 2122×10^{-6} which is a lower value than the sum of the molar susceptibility values of Cu(II)-glygly and ferricyanide (see Table I). These magnetic and EPR data are not in accord with this mechanism. In another experiment the 1:1 Cu(II)-glygly·ferricyanide complex was diluted by adding excess diamagnetic cobalticyanide. The molar susceptibility of this mixture is close to the sum of the molar susceptibility values of Cu(II)-glygly and ferricyanide and they are shown in Table I. The EPR spectra of 1:1 Cu(II)-glygly·ferricyanide complex were also measured in presence of increasing concentrations of diamagnetic cobalticyanide and are given in Fig. 2. If the Cu(II) is oxidized to Cu(III), the dilution with cobalticyanide does not increase the EPR signal and molar susceptibility value. Thus, the above experiments completely ruled out the second mechanism of oxidation of Cu(II) to Cu(III) by ferricyanide.

The disappearance of the EPR signal of Cu(II) in Cu(II)-glygly·ferricyanide complex at liquid nitrogen temperature, the low value of molar susceptibility of this complex, and the increase in the value of molar susceptibility and EPR signal of this complex after diluting it with excess of diamagnetic cobalticyanide complex can be interpreted in terms of the third mechanism which involves antiferromagnetic coupling between Cu(II) and Fe(III) through cyanide bridge as $Cu^{II} \leftarrow N{\equiv}C{-}Fe^{III}$. The Cu(II)-glygly has two coordinated water molecules [9]. One of the coordinated water molecules in Cu(II)-glygly can be replaced by coordination of nitrogen of one of the bonded cyanide of ferricyanide with the formation of cyanide bridged Cu(II)-glygly·ferricyanide complex as given above. The EPR data indicate that the ground state of Cu(II)-glygly·ferricyanide complex is diamagnetic at liquid nitrogen. The magnetic and EPR data at room temperature indicate that the low energy excited

levels of the complex are populated at room temperature and they are responsible for the paramagnetism.

The Cu(II)-glygly•ferricyanide complex is a model for the low spin cyanide complex of fully oxidized cytochrome a_3 (Fe^{III}–CN–Cu^{II}). Furthermore, there may be a similar bridged complex formation between Cu(II) of galactose oxidase and ferricyanide, which is responsible for the disappearance of the EPR signal of Cu(II).

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