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Effect of Deprotonation of the Coordinated Water Molecule on Properties of Copper(II) Complex with Glycylglycine and H_2O

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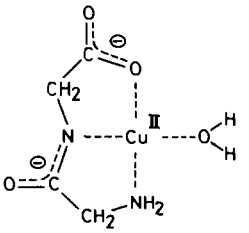
Abstract

We studied properties of the copper(II) complex with glycylglycine ([GlyGlyCu$^{II}H_2O$]) in aqueous solution using potentiometric titration, electron spin resonance (ESR) spectroscopy, and polarography, to see the effect of deprotonation at the coordinated water molecule. Deprotonation gives rise to a copper(II) complex with OH^- ([GlyGlyCu$^{II}OH^-$]$^-$). The pK_a value was 9.31 from potentiometric titration and 9.10 from ESR spectroscopy. Polarographic data, however, showed that this value was much higher. Although deprotonated complex with OH^- was produced above pH 8 in the solution, it was reduced only above pH 10.5. The difference in the complex species involved in the bulk solution and reduced at the electrode was ascribed to the equilibria, which made the minor complex species with H_2O having a higher redox potential to be reduced predominantly at the surface of the electrode. The deprotonation of the water molecule bound to the copper(II) complex brought about a negative shift in the redox property of the complex. Therefore, deprotonation resulted in a decreased ability of the complex to accept electrons.

Introduction

There is now much interest in the mechanism of electron transfers involving multicopper oxidases like laccase and redox agents. This enzyme catalyzes the oxidation of a variety of substrates and uses molecular oxygen as the sole electron acceptor to form water [1]. The electron transfer from the substrate to the molecular oxygen is mediated by three kinds of copper atoms in the enzyme, which are designated as type 1, 2, and 3 copper according to their properties [2]. An initial reduction of type 1 copper is followed by a reduction of type 2 and then an intramolecular electron transfer to the type 3 site, where the molecular oxygen is bound and its reduction to water is thought to proceed [1, 3].



[GlyGlyCu$^{II}H_2O$]

Binding of a hydroxide ion at the type 2 copper may regulate the electron transfer, since at high pH the inhibition of the enzyme catalysis couples with formation of the type 2 Cu^{II}–OH^- complex. This type 2 copper is not reduced until the OH^- ion has been eliminated either by dissociation or by protonation [3]. Although differences in the redox potential are expected, the redox properties of the enzyme have not been established in detail. This is also true for many copper(II) complexes of low molecular weight having a H_2O or a OH^- as a monodentate ligand. It is of interest to know whether deprotonation of the coordinated water molecule triggers electron transfer.

Here, we studied the trigger mechanism using a model copper(II) complex having a tridentate ligand of glycylglycine and a monodentate ligand of H_2O. With increased pH of the solution, the coordinated water becomes deprotonated to give the complex with OH^- [4]. We investigated the properties of this complex with H_2O and those with OH^- in aqueous solutions as a function of pH.

Experimental

Materials

Reagents were of the highest grade commercially available, and distilled deionized water was used throughout.

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Preparation of Copper(II) Complex with Glycylglycine and H_2O, ([GlyGlyCu$^{II}H_2O$])

To a stirred solution of $Cu(ClO_4)_2 \cdot 6H_2O$ (3.71 g, 10 mmol) in 30 cm^3 of water was added glycylglycine (1.32 g, 10 mmol) in 20 cm^3 of water. The solution was neutralized by the addition of sodium hydroxide, and then 30 cm^3 of dimethyl formamide was added. The mixture was evaporated to half of its volume under reduced pressure. After being left overnight, dark blue crystals of [GlyGlyCu$^{II}H_2O$] were collected by filtration, washed with ethanol, and dried *in vacuo*. *Anal.* Found: C, 22.72; H, 3.92; N, 13.20. Calc. for $[(C_4H_6O_3N_2)Cu(H_2O)]$: C, 22.70; H, 3.81; N, 13.24%. This procedure gives rise to a single product of the monohydrate and not a complex of trihydrate, which was reported by Martel *et al.* [5].

Potentiometric Titration

An aqueous solution of glycylglycine and copper(II) perchlorate (1.0×10^{-2} mol dm^{-3}) was titrated with 1.0 mol dm^{-3} sodium hydroxide under a nitrogen atmosphere. Enough sodium perchlorate was added to maintain the ionic strength at 1.0. Changes in pH were followed using a glass electrode and a Horiba Model F-8 pH meter calibrated in terms of hydrogen ion concentration. The system was standardized before and after the experiment with Wako standard buffer solutions (pH 6.86 and 9.18). The temperature of the solution was controlled at 25.0 ± 0.1 °C by hot water circulating from a Neslab Model RTE-8 thermostat to the water jacket of the cell.

ESR Measurements

ESR spectra were obtained at 9.25–9.35 GHz with a JOEL Model JES-ME-1X ESR spectroscopy at 77 K. The g values were calibrated with Mn^{2+} sealed in a quartz capillary tube as the external standard. Samples were dissolved in aqueous solution containing 0.1 mol dm^{-3} of sodium perchlorate and the pH was adjusted with a few drops of concentrated perchloric acid and sodium hydroxide. The final concentration of the complex ranged from 5 to 6×10^{-3} mol dm^{-3}.

Polarographic Measurements

A Fuso Model 312 potentiostat was used for polarographic measurements. A fiber-plug-type saturated calomel electrode (SCE) of the Yanagimoto Model MR-P2 electrode was invariably used. Thus, the potentials given in this paper are always with respect to an SCE. A Riken Denshi Model F-43P X-Y recorder was used for recording the polarograms. The temperature of the cells was controlled at 25.0 ± 0.1 °C in the same way as for the potentiometric titration. The dropping mercury electrode had a column height of 70 cm and a mass flow rate of 2.06 mg s^{-1}. The polarographic potential variations were followed with a Takeda Riken Model TR-6841 digital voltmeter. The concentration of the complex was 1.0×10^{-3} mol dm^{-3}. All solutions were deaerated in a stream of highly pure (99.9999%) nitrogen gas.

Results and Discussion

Potentiometric Titration of Copper(II) Complex with Glycylglycine and H_2O

The copper(II) complex with glycylglycine is obtained by the addition of the dipeptide to an aqueous solution containing an equimolar amount of Cu^{2+} ion above pH 6 [4–7]. As a crystal, the complex has square planar geometry by X-ray crystallographic analysis [8]. The copper(II) atom is coordinated by a terminal amino nitrogen, a deprotonated amide nitrogen, a carboxylate oxygen of the ligand, and an oxygen of a water molecule [8, 9]. In aqueous solution, the complex structure depends on the equilibria present in the solution. The equilibrium constants for complex formation and the subsequent ionization of the amide nitrogen to give [GlyGlyCu$^{II}H_2O$] have already been reported [4–7]. However, further deprotonation at the coordinated water molecule has not been studied in detail. Some authors have reported the equilibrium constant for such deprotonation [4, 7], but their values do not agree. This is probably because their use of a dilute base solution was not suitable for obtaining the equilibrium constant for the deprotonation, which occurs at a higher pH than complex formation. In this study, the complex solution was titrated with concentrated sodium hydroxide solution (1.0 mol dm^{-3}) at a high ionic strength of 1.0 with sodium perchlorate. This condition prevented precipitation or aggregation of the complex even at high pH.

The titration curve of the solution is shown in Fig. 1; two relatively flat zones were found. The first buffer zone around pH 4 shows the formation of [GlyGlyCu$^{II}H_2O$] with a loss of two protons, which agrees well with the results already in the literature [4–7]. The second relatively flat buffer zone and a weak inflection were in the higher pH region where the deprotonation of the coordinated water takes place [9, 10]. At this region of high pH, the following two equilibria were considered to be present in the solution:

$$2[\mathrm{GlyGlyCu^{II}H_2O} \overset{K_1}{\rightleftharpoons} [\mathrm{GlyGlyCu^{II}}(\mathrm{OH^-})\mathrm{Cu^{II}GlyGly}]^- + \mathrm{H^+} + \mathrm{H_2O} \quad (1)$$

$$[\mathrm{GlyGlyCu^{II}H_2O}] \overset{K_2}{\rightleftharpoons} [\mathrm{GlyGlyCu^{II}OH^-}]^- + \mathrm{H^+} \quad (2)$$

The first equilibrium constant of K_1 shows formation of a μ-hydroxo binuclear complex, and the second

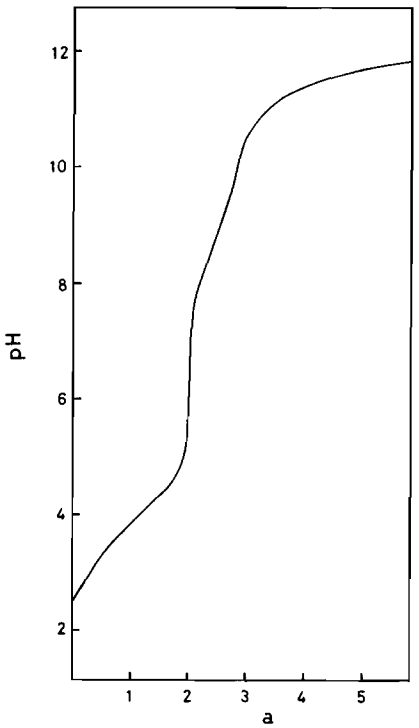

Fig. 1. Potentiometric titration curve for glycylglycine–cupric perchlorate system at 25.0 ± 0.1 °C and μ = 1.0 with $NaClO_4$: [copper(II)] = [glycylglycine] ≈ 1.0×10^{-2} mol dm^{-3}. a = mol of standard NaOH added per mol of ligand.

equilibrium constant of K_2 shows formation of a mononuclear complex with OH^-. From the titration results, pK_1 and pK_2 were calculated to be 5.06 and 9.31, respectively. These values were relatively smaller than those already reported [4, 7], the mean values of which were 5.49 and 9.46, respectively. These differences may be ascribed to different experimental conditions. The obtained pK_1 and pK_2 values show that the deprotonated complex of $[GlyGlyCu^{II}\cdot OH^-]^-$ becomes the major species in solution above pH 9.0. At pH 10.0, for instance, more than 95% of the cupric atoms were present as the deprotonated complex with OH^- including the μ-hydroxo complex.

Effect of Deprotonation upon ESR Spectra

ESR spectra of copper(II) complexes are sensitive not only to the coordinating atoms but also to the net charge of the complexes [11]. In general, the $g_{\parallel}$ value tends to decrease and the $|A_{\parallel}|$ value increases with the rise in the negative charge of the ligand, even though the numbers and kinds of the ligand atoms are the same. Thus, the deprotonation of the coordinated water molecule in the complex in question should be observed through changes in the ESR spectra, which were recorded at several pH values and are shown in Fig. 2.

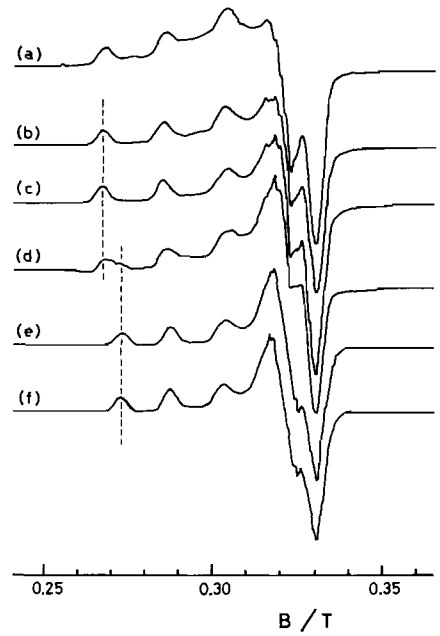

Fig. 2. ESR spectra of frozen (77 K) aqueous solution of the copper(II) complex with glycylglycine at (a) pH 4.7; (b) pH 5.3; (c) pH 7.2; (d) pH 9.0; (e) pH 9.9; and (f) pH 12.8. Instrumental settings were 6 mW for microwave power, modulation amplitude of 2.0 gauss, time constant of 0.3 s, and a sweep rate of 0.025 T min^{-1}.

The signal observed for the complex solution at pH 5.3 can be assigned to the signal $[GlyGlyCu^{II}\cdot H_2O]$, since complex formation is almost completed at this pH. At pH 4.7, where the complex formation is under way, a broad signal which suggests the contamination with $[H^+GlyGlyCu^{II}(H_2O)_2]^+$ was observed. A very small signal that appeared at 0.256 T was attributed to free Cu^{2+} ion involved as a very minor cupric species. When the pH of the solution was increased from 7.2 to 9.9, the spectrum changed to a new one that did not change further with additional increases in the pH. Gould and Mason [12] reported similar signals, but they did not explain these changes in the signal. Since the coordinated water molecule becomes deprotonated in this pH region, we think that this change is derived from this deprotonation. Therefore, the new signal can be assigned to the signal of $[GlyGlyCu^{II}OH^-]^-$. Although the deprotonation brings about apparent changes seen in Fig. 2, only a small effect is observed on the ESR parameters of $g_{\parallel}$ and $g_{\perp}$ ($g_{\parallel H_2O} = g_{\parallel OH^-} = 2.235 \pm 0.005$; $g_{\perp H_2O} = g_{\perp OH^-} = 2.050 \pm 0.005$). On the other hand, the hyperfine coupling constant $|A_{\parallel}|$ changed markedly, decreasing from 19.0 to 15.2 × 10^{-3} cm^{-1} with deprotonation. The $|A_{\parallel}|$ value was reported to increase when the negative charge of the ligand increases [11]. What we observed here with deprotonation of the coordinated water molecule was quite the opposite.

At pH 9.0, where the solution is near the midpoint of the deprotonation process, the three kinds of complexes shown in eqns. (1) and (2) may be involved. In the ESR spectrum, however, only two signals, which we assigned to the signal of the complex with H_2O and to that of the complex with OH^-, were observed, with a marked decrease in the signal intensity. The magnitude of the signal was less than one-third of the intensity of the signal observed at pH 7.2 or 9.9. In Fig. 2, signals of the same size were recorded with increasing the amplitude of the ESR spectroscopy. The decrease in the signal intensity may be explained by the deprotonated product of the μ-hydroxo binuclear complex being ESR-silent. Since many other binuclear copper(II) complexes with a μ-hydroxo bridge are reported to have a strong antiferromagnetic interaction between the two copper nuclei [13], a similar effect is not unexpected in this case, which would make the ESR spectrum silent. During this deprotonation, the decrease in the concentration of the complex of $[GlyGlyCu^{II}H_2O]$ was evaluated from two signal intensities at 0.268 and 0.272 T, where signals of $[GlyGlyCu^{II}H_2O]$ and $[GlyGlyCu^{II}OH^-]^-$ appear, respectively; this is shown in Fig. 3. From this curve, the apparent pK_a value for deprotonation was obtained as 9.10, which is consistent with the results obtained from the potentiometric titration. That the spectrum does not change further above pH 9.9 suggests that the deprotonation giving rise to $[GlyGlyCu^{II}OH^-]^-$ is already complete at this pH, and that the complex species with two OH^- ions proposed by some authors [4] is not present in a detectable amount under these experimental conditions.

These ESR results show that the deprotonation of the coordinated water molecule takes place in the pH region from 8.5 to 9.5, and that above pH 10, the complex of $[GlyGlyCu^{II}OH^-]^-$ is the sole complex species in solution.

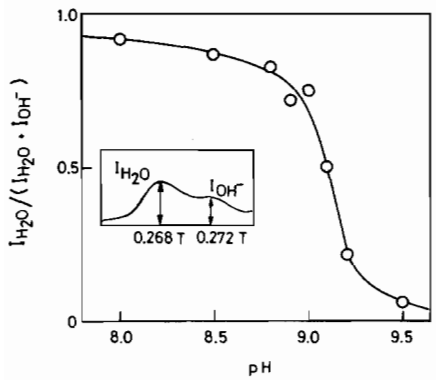

Fig. 3. pH-Dependence of $I_{H_2O}/(I_{H_2O}+I_{OH^-})$, where I_{H_2O} and I_{OH^-} represent ESR signal intensities for $[GlyGlyCu^{II}H_2O]$ (0.268 T) and $[GlyGlyCu^{II}OH^-]^-$ (0.272 T), respectively. The inset shows the ESR signals in the low-field edge of the perpendicular region.

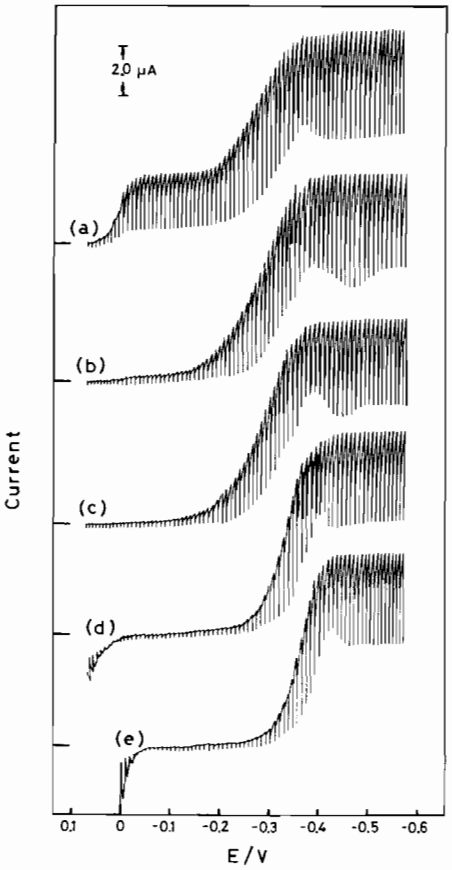

Fig. 4. d.c.-Polarograms of the copper(II) complex with glycylglycine in aqueous solution at (a) pH 5.3; (b) pH 6.9; (c) pH 8.5; (d) pH 10.5; and (e) pH 11.5; at 25.0 ± 0.1 °C, and μ = 0.1 with $NaClO_4$.

Effect of Deprotonation upon Polarograms

d.c.-Polarograms of the copper(II) complex with glycylglycine were recorded at several pH values and are shown in Fig. 4. At pH 5.3, two reduction waves were observed. The first wave at anodic potential corresponds to reduction of free Cu^{2+} ions, since free Cu^{2+} ions had a cathodic wave at the same potential. By comparing the limiting current of this wave to that for a solution containing only free Cu^{2+} ions, we calculated that 33% of the total cupric atoms were reduced as the free Cu^{2+} ions, although in the bulk solution less than 10% of the cupric atoms were present as such ions. The second wave can probably be attributed to reduction of the copper(II) complex with glycylglycine.

At pH 6.9, the first wave disappeared completely and the second wave increased to the height corresponding to two-electron uptake, which was confirmed by controlled-potential coulometry. The pH-dependence of the apparent half wave potential, $E_{1/2}$, of the second cathodic wave is shown in Fig. 5. From pH 6.9 to 8.5, this cathodic wave was almost unchanged, and the $E_{1/2}$ changed only slightly. Above

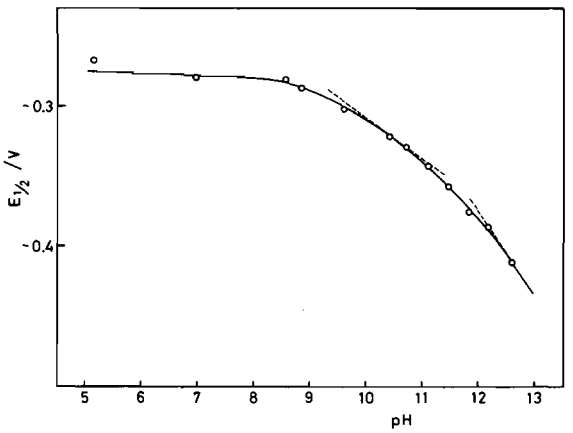

Fig. 5. Plot of $E_{1/2}$ *vs.* pH of the solution for the copper(II) complex with glycylglycine.

pH 8.5, however, this wave shifted progressively towards negative potentials and the $E_{1/2}$ decreased further with the rise in the pH. Although cathodic waves appear to be irreversible especially at lower pH conditions, each wave seemed to be made up of reversible cathodic waves of the complex species present in the solution at equilibrium. At pH 10.5, for instance, a plot of $\log(i/(i_d - i))$ *vs.* electrode potential was a straight line with an inverse slope of 34 mV, suggesting that polarographic two-electron reduction is almost reversible.

When the complex of $[GlyGlyCu^{II}H_2O]$ is reduced at the surface of the electrode by two-electron transfers to metallic copper, the GlyGly ligand, having a deprotonated amide nitrogen, can neither be bound tightly to the metallic copper nor be liberated wholly to the bulk solution as the deprotonated dipeptide, since in the absence of the cupric ion the dipeptide is reported not to be ionized even in strongly alkaline solution [1]. This is also the case for the OH^- ion in $[GlyGlyCu^{II}OH^-]^-$. Therefore, reduction of $[GlyGlyCu^{II}H_2O]$ will be coupled with one-proton transfer and that of $[GlyGlyCu^{II}OH^-]^-$ with two-proton transfers, one proton to the amide nitrogen and the other to the OH^-.

$$\left.\begin{array}{l}[GlyGlyCu^{II}H_2O] + H^+ \xrightarrow{2e^-} \\ [GlyGlyCu^{II}OH^-]^- + 2H^+ \xrightarrow{2e^-}\end{array}\right\} \rightarrow Cu^0 + H^+GlyGly + H_2O \quad (3)$$

As shown in the potentiometric titration, $[GlyGlyCu^{II}H_2O]$ is formed above pH 4.5. At pH 6.0, for example, more than 99% of the cupric atoms were present as this complexed species. If the main complex species reduced at the electrode were the same as the major species in the solution, the complex with H_2O at pH 6.0 should be reduced accompanied by one-proton uptake. However, the $E_{1/2}$ was fairly constant from pH 5.3 to 8.5, which does not suggest proton transfer accompanying the reduction. This means that the reduced complex species in this pH region is not $[GlyGlyCu^{II}H_2O]$ but probably $[H^+GlyGlyCu^{II}(H_2O)_2]^+$, which is a very minor complex species present in the solution at equilibrium. The shift of $E_{1/2}$ with pH is given by

$$-(\Delta E_{1/2})/\Delta pH = 0.05915(P/n) \quad (4)$$

where n and P are the number of electrons and protons involved in the electrode reaction, respectively [14].

When the pH of the solution was increased from 8.5 to 10.5, $E_{1/2}$ shifted to negative potentials and the slope of the tangent of the curve in Fig. 5 (the slope is designated $-(\Delta E_{1/2})/\Delta pH$) reached 30 mV. This shows that the reduction is accompanied by one-proton uptake (eqn. (4)) and accordingly that the reduced complex species here is $[GlyGlyCu^{II}H_2O]$.

Above pH 10.5, the cathodic wave shifted to more negative potentials and the slope of $-(\Delta E_{1/2})/\Delta pH$ increased further to 59 mV. In addition, an anodic wave was seen at potentials higher than −0.1 V that increased with the additional rise in the pH. These results suggest that in this region of high pH, the complex with OH^- including the μ-hydroxo complex is the redox active species. On the polarograms, characteristics of the μ-hydroxo complex are not seen. This complex seems to have an intermediate character between the complex with H_2O and that with OH^-. That the slope of $-(\Delta E_{1/2})/\Delta pH$ approaches 59 mV at pH 12.6 shows that the reduction tends to be accompanied by two-proton transfers (eqn. (4)). Therefore, it seems that only at this pH does $[GlyGlyCu^{II}OH^-]^-$ become the sole complex species reduced at the electrode, although in the bulk solution this complex is readily formed at pH 10.0, as shown in the results of titration and ESR spectra.

These differences in the complex species present in the bulk solution and reduced at the surface of the electrode may arise from the equilibria shown in eqns. (1) and (2). A minor complex species in solution can be reduced preferentially at the electrode, if it has higher redox potential than major complex species. Therefore, the redox potential of the complex seems to decrease in the following order: free Cu^{2+} ion > $[H^+GlyGlyCu^{II}(H_2O)_2]^+$ > $[GlyGlyCu^{II}H_2O]$ > $[GlyGlyCu^{II}OH^-]^-$. A comparison between the complex species in the bulk solution and that reduced at the electrode is given in Fig. 6.

In conclusion, the reduced complex species at the surface of the electrode is not compatible with the major complex species involved in the bulk solution, if there are other complex species at equilibrium having higher redox potential. The effect of deprotonation of the coordinated water molecule is large in the redox potential of the complex. Deprotonation of the coordinated water molecule can result in an

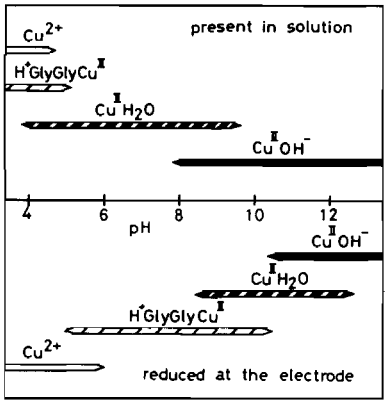

Fig. 6. Comparison of complex species present in the aqueous solution and those reduced at the surface of the electrode as a function of pH. Cu^{2+}, $H^{+}GlyGlyCu^{II}$, $Cu^{II}H_2O$, and $Cu^{II}OH^{-}$ represent free Cu^{2+} ion, $[H^{+}GlyGlyCu^{II}(H_2O)_2]^{+}$, $[GlyGlyCu^{II}H_2O]$, and the deprotonated complex with OH^{-} including the μ-hydroxo complex.

on–off regulation for electron transfers of the copper(II) complexes; probably a similar effect will be found with multi-copper enzymes.

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