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Studies on Heterobinuclear Copper-Zinc Complexes of Amino Acids as Biomimic Systems of Superoxide Dismutase

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Superoxide dismutase (SOD) contains two metal ions Cu(II) and Zn(II), active sites being bridged by the imidazolate anion. A model system for this enzyme has been investigated by attempted syntheses of copper-zinc complexes of amino acids $[CuZn(L_1)_4(H_2O)_2]$ where L_1 = glycine and $[CuZn(L_2)_3(H_2O)_2]$ where L_2 = histidine. The characterization of these compounds was carried out by conductance measurements, electronic, electron paramagnetic resonance (EPR) spectroscopy, fourier transform infrared spectra (FT-IR) and cyclic voltammetry along with the catalytic decomposition of hydrogen peroxide. From the above studies, it is interesting to note that the complex $[CuZn(his)_3(H_2O)_2]$ alone forms a mixed metal complex, which *mimics* the native enzyme both structurally and spectroscopically whereas glycine complexes form only the mechanical mixture of CuL_1 and ZnL_1. The peculiar coordination behaviour of histidine is its ability to act as a bridging ligand between the two metal atoms through the nitrogen atoms of imidazolate anions as observed in the native superoxide dismutase enzyme.

Key Words: Superoxide dismutase, Model complex of Cu-Zn SOD, Heterobinuclear Cu-Zn complexes, Histidine.

INTRODUCTION

Superoxide dismutase (SOD) is a dimeric metal-binding enzyme responsible for the dismutation of toxic superoxide to hydrogen peroxide and oxygen in cells[1]. Copper-zinc superoxide dismutase (SOD) has been widely considered a major intracellular antioxidant enzyme in mammals. There are two CuZnSODs encoded by two different genes found in eukaryotes: intracellular CuZnSODs (icCuZnSODs) and extra-cellular CuZnSODs (ecCuZnSOD). icCuZnSOD is present in the cytoplasm and nucleus, whereas ecCuZnSOD is found in the extracellular matrix of tissues such as lympha and plasma[2]. icCuZnSOD is a basic form and is essential for oxygen respiration in animals based on the high conservation of the entire molecule. It is known that 90 % of the total superoxide dismutase (SOD) activity is due to icCuZnSOD in eukaryotes[3]. CuZnSOD is one of the most important metalloenzymes in the first line of defense against oxidative stress. CuZnSOD counteracts the effects of these oxidizing substances and is involved in the pathophysiology of human diseases such as atherosclerosis, rheumatoid arthritis and some tumors[4,5]. Although many SOD model compounds are reported[6-9], their structures are quite different from those of the native enzyme. Also, the method of preparation of them was difficult and involves many chemicals. The properties of imidazolate-bridged heterobinuclear model complexes having a pair of metal atoms *viz.*, copper(II)-zinc(II) have been described[10-12]. They consist of two independent species *viz.*, copper(II) and zinc(II) complexes respectively, coordinated by an imidazolate bridge between the copper and zinc metal atoms. Thus copper-zinc heterobinuclear complexes with amino acid ligands (glycine, histidine) have been attempted for synthesis in the present work. Among the amino acids chosen, the histidine complexes are expected to be more interesting as it is involved in the native enzyme superoxide dismutase. Histidine bridges Cu and Zn through the histidine moiety[13]. Zinc is expected to regulate the ligational behaviour of the counter nitrogen atom to the copper center and thereby induce the required electronic environment[14-16]. This regulation is necessary to bring the disproportionation of the superoxide ion within the required physiological rates[17,18]. In this paper, we report the investigations of the Cu-Zn complexes with glycine and histidine by a simple method. The discrete complexes are compared with the mechanical mixtures of the complexes to identify the formation of discrete complexes. To the best of our knowledge, the present method which we adopted in this study is a simple method involving fewer chemicals than the other methods reported so far in the literature. Enzymes (especially the

metalloenzymes) are the catalysts of processes occurring in living systems. From the point of view of the catalysis, the active centre of an enzyme is a metal complex. In the most cases the active centre formed by donor atoms located in the side chains of the amino acids, which are incorporated in the polypeptides forming the enzymes. To approach the catalytic activity of an enzyme - mimicking the enzyme, we have a possibility of mimicking the effect of the enzyme by metal complexes, which have similar or closely similar activity in the chosen reaction. Thus the decomposition of hydrogen peroxide with $[CuZn(gly)_4(H_2O)_2]$ complex and $[CuZn(his)_3(H_2O)_2]$ complex were also discussed in the present study.

EXPERIMENTAL

Copper carbonate hydroxide (Aldrich), zinc carbonate hydroxide (Aldrich) and other chemicals of laboratory reagent grade were used.

Preparation of complexes

Preparation of $CuZn(gly)_4(H_2O)$: A calculated amount of glycine was dissolved in hot distilled water and mixed with copper carbonate hydroxide (1.13 g, 0.50 mol) and zinc carbonate hydroxide (0.88 g, 0.50 mol) separately. The solutions were then heated on a water bath and dried. The obtained bis(glycinato)copper(II) complex and bis(glycinato)zinc(II) complex were kept in a dessicator for drying. Equal amounts of these complexes were mixed well by grinding them together for 1 h to get a mixture and refluxed with benzene over a water bath. The solution was then filtered and dried. The obtained yield was 80 %.

Preparation of $CuZn(His)_3(H_2O)_2$: Calculated amounts of histidine was dissolved in hot distilled water and mixed with copper carbonate hydroxide (0.85 g, 0.80 mol) and zinc carbonate hydroxide (0.44 g, 1.10 mol) separately. The solutions were then heated on a water bath. The obtained *bis*(histidinato)copper(II) complex and *bis*(histidinato)zinc(II) complex were kept in a dessicator for drying. The yield obtained was 60 %.

Equal amounts of these two complexes were mixed well by grinding them together for 1 h to get a mixture. The mixture was refluxed with benzene over a water bath. The solution was then filtered and dried. The yield obtained was 70 %.

Conductance of the copper-zinc mixed metal complex of amino acid (histidine and glycine) was studied using P.I.Co Conductivity Bridge. The electronic spectra of the complexes in the visible region were recorded with the samples distributed in Nujol mull using Perkin Elmer UV-visible spectrophotometer. The EPR spectrum of the complexes was recorded in powder form at room temperature using Varian-E-112x band spectrometer. The infrared spectra of the complex were recorded in solid state in KBr medium using Perkin Elmer FT-IR spectrometer. The cyclic voltammogram of the complex was recorded using RDE 0018 analytical cell.

RESULTS AND DISCUSSION

The heterobinuclear complex of copper(II) and zinc(II) metal ion was prepared by mixing the complex CuL_2 and $ZnL_2{\cdot}2H_2O$ in 1:1 mole ratio in benzene medium, where L = glycine and histidine. The percentage of metal ion and nitrogen present in the complex was estimated quantitatively. The analytical data of the complexes was given in the Table-1.

Conductance of copper-zinc glycine and histidine complexes: The conductances of both Cu(II) and Zn(II) complexes were done at different molar concentration ranging from 2×10^{-4}-2×10^{-3} using the conductivity bridge. A comparative study of conductance of complex and its mechanical mixture were done under similar conditions of concentration. The plot of λ_m *versus* $\sqrt{C}$ was drawn for each complex is shown in Fig. 1.

Bis(glycinato)copper(II) complex shows lower conductance and *bis*(glycinato)zinc(II) complex shows higher conductance. The $CuZn(gly)_4(H_2O)$ complex and its mechanical mixture show similar behaviour of conductance as shown in Fig. 1(A). Thus the study of conductance of copper zinc mixed metal complex of glycine amino acid shows that the isolated complex $CuZn(gly)_4(H_2O)$ may be a mechanical mixture of the complexes of *bis*(glycinato)copper(II) and *bis*(glycinato) zinc(II). *Bis*(histidinato)copper(II) complex shows lower conductance and *bis*(histidinato)zinc(II) complex shows higher conductance as shown in Fig. 1(B). The discrete complex and its mechanical mixture show different behaviour in conductance and reveals that the nature of the discrete complex $[CuZn(his)_3(H_2O)_2]$ is entirely different from its mechanical mixture. Thus conductivity study results propose the formation of the complex in the case of $[CuZn(his)_3(H_2O)_2]$.

Electronic spectra: The visible spectra of the refluxed complex and its mechanical mixture were recorded in Nujol mull. The refluxed complex and its mechanical mixture showed a broad absorption band over a range of 600-750 nm. It is reported[19] that the native enzyme Cu-Zn-SOD shows a λ_{max} of 680 nm, at pH 6.1 and 0.05 mM concentration and hence the aqueous solutions of the complex $[CuZn(gly)_4(H_2O)_2]$, $[CuZn(his)_3(H_2O)_2]$ and its mechanical mixtures were prepared in the same pH and concentration, using potassium phosphate buffer and the nature of absorptions of these solutions were measured using a spectrophotometer. The plot of absorbance *versus* λ has been drawn for the complexes as shown in Fig. 2.

The curves of a and b of Fig. 2(A) are similar in behaviour suggesting that the complex $[CuZn(gly)_4(H_2O)_2]$ and its mechanical mixture are similar and the λ_{max} is 640 nm indicating no interaction between the two metal ions[20]. Whereas the curves of a and b of Fig. 2(B) indicate that complex $[CuZn(his)_3(H_2O)_2]$ and its mechanical mixture has different

TABLE-1

Complexes	Colour	Colour of the mechanical mixtures	Cu (%)		Zn (%)		N (%)	
			Found	Calcd.	Found	Calcd.	Found	Calcd.
$[CuZn(gly)_4(H_2O)_2]$	Blue	Blue	12.36	13.79	13.65	14.18	12.04	12.15
$[CuZn(his)_3(H_2O)_2]$	Deep blue	Grey	9.95	10.08	10.22	10.37	17.64	19.98

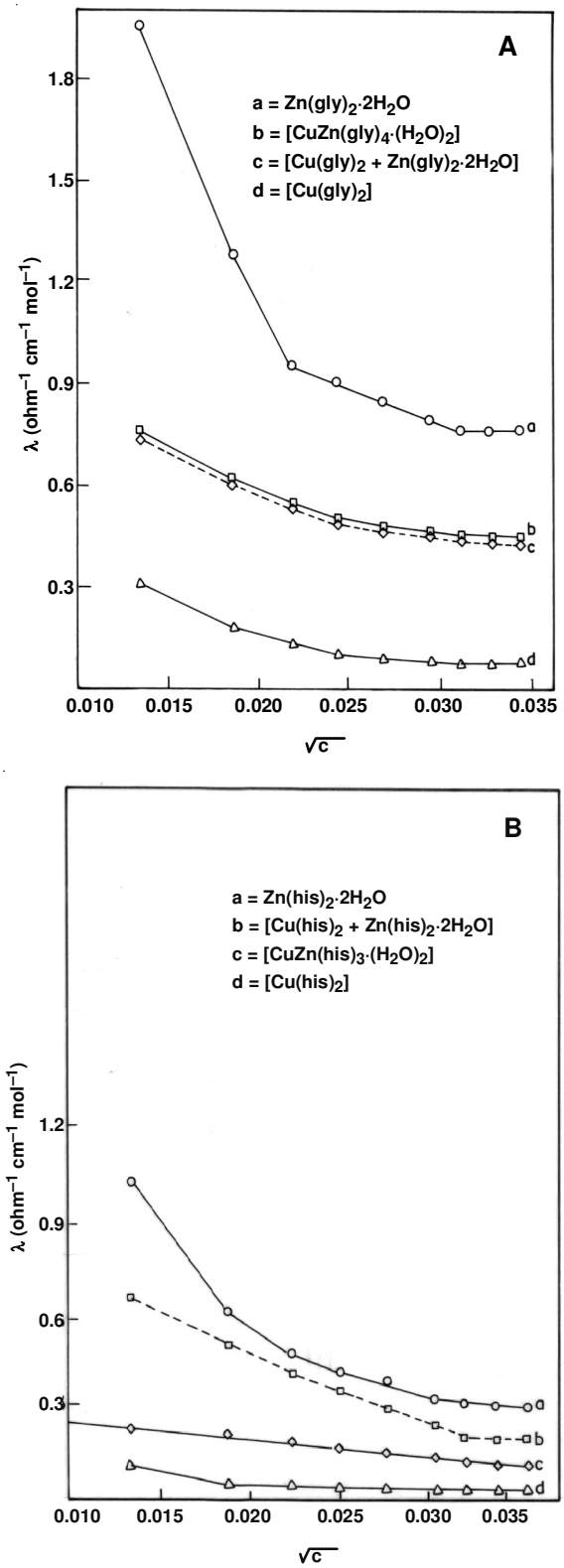

Fig. 1. (A) Molar conductance plot of glycine complexes (B) molar conductance plot of histidine complexes

λ_{max} at 710 and 650 nm, respectively. Thus the electronic spectral data propose that amino acid histidine coordinates with copper(II) and zinc(II) metal ions and forms a discrete complex $[CuZn(his)_3(H_2O)_2]$, which is totally different from its mechanical mixture whereas glycine does not favour such a complex.

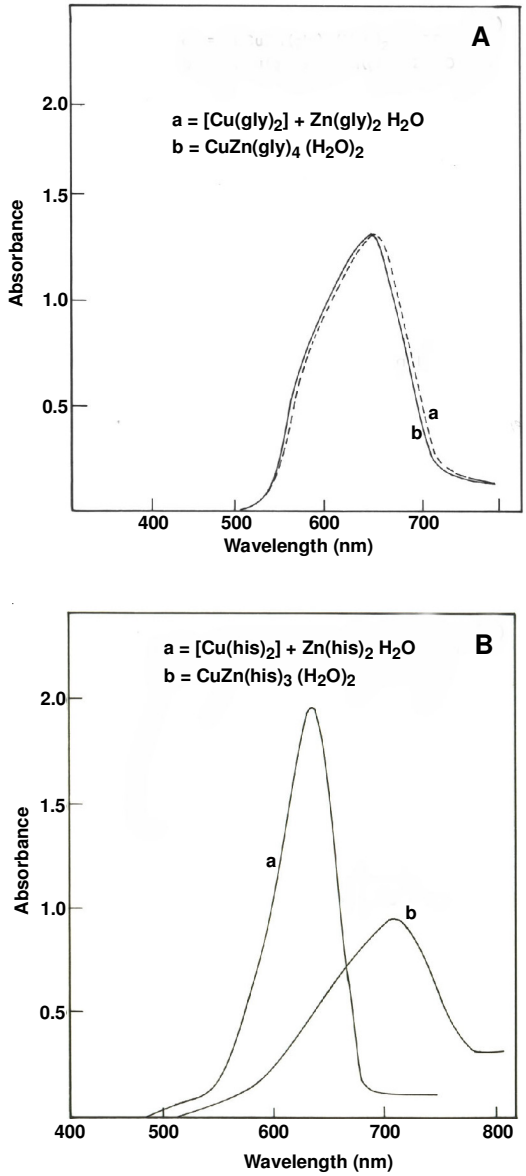

Fig. 2. (A) Absorption spectra of glycine complex (B) absorption spectra of histidine complex

EPR spectral studies: The EPR spectral pattern was shown in the Figs. 3 and 4. The 'g' value was calculated using the formula,

$$h\nu = g\beta H$$

where h = Planck's constant, 6.626×10^{-34} joule s, ν = frequency in Hz, g = gyromagnetic ratio (no unit), β = Bohr magneton, 9.274×10^{-24} J T^{-1} and H = field strength.

The calculated 'g' value for the complex $[CuZn(gly)_4(H_2O)_2]$ and its mechanical mixture are the same *i.e.*, g = 2.1993 and there is no shift in peak positions of the spectra of these complexes. The equal g values implies that the complex $[CuZn(gly)_4(H_2O)_2]$ and its mechanical mixture are similar. The calculated 'g' value for the complex $[CuZn(his)_3(H_2O)_2]$ and its mechanical mixture are 2.0936 and 2.1096, respectively. The different g values implies that the complex $[CuZn(his)_3(H_2O)_2]$ is different from its mechanical mixture. The EPR parameter (g = 2.0936) of the complex $[CuZn(his)_3(H_2O)_2]$ is comparable[21,22] with that of the active

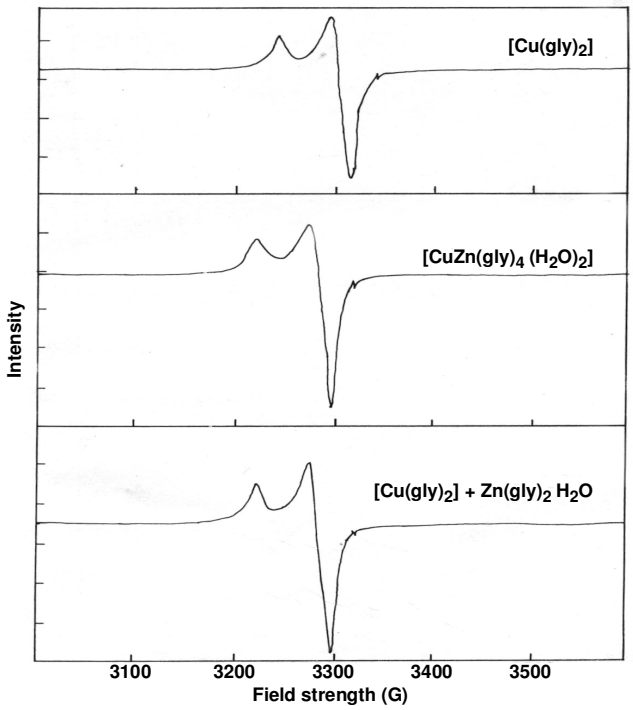

Fig. 3. EPR spectra of glycine complex

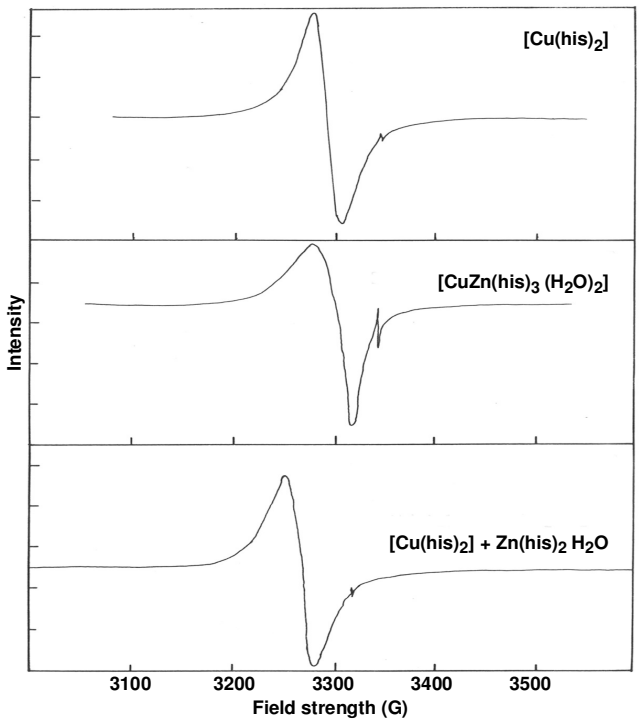

Fig. 4. EPR spectra of histidine complex

center of superoxide dismutase (g = 2.0820). Thus histidine forms a discrete copper-zinc heterobinuclear complex, which *mimics*[23] the native enzyme superoxide dismutase. The broadness of signal b in Fig. 4 suggests that the copper-copper interaction is more in the case of discrete complex than in the mechanical mixture. Thus the EPR spectral data gives the evidence for the formation of heterobinuclear complex $[CuZn(his)_3(H_2O)_2]$.

Infrared spectra: The infrared spectra of mixed metal complex of histidine alone were recorded using KBr pellets. The infrared spectrum of $[CuZn(his)_3(H_2O)_2]$ is shown in Fig. 5. The IR spectra of the complexes support the existence of the coordination bonds and the formation of metal complexes with the suggested structure. The band with a maximum at 3025 cm^{-1} (νNH), characteristic for imidazol, disappeared in the spectra of the metal complexes, indicating the presence of the imidazolate ion[24]. In case of Cu-Zn-SOD, one of the histidine molecules acts as bridging ligand by coordinating through nitrogen atoms of imidazole anion[25]. The free histidine shows ν(C=N) at 1900-1500 cm^{-1}, but in the complex $[CuZn(his)_3(H_2O)_2]$ showed ν(C=N) at 1498 cm^{-1}. This indicated that, as in the case of Cu-Zn-SOD histidine is bridging between the metal ions copper(II) and zinc(II) through the nitrogen atoms of imidazolate anion and cause a shift in ν(C=N) to 1498 cm^{-1}. Thus the infrared spectrum also suggested the formation of heterobinuclear complex of copper(II) and zinc(II) metal ions with histidine.

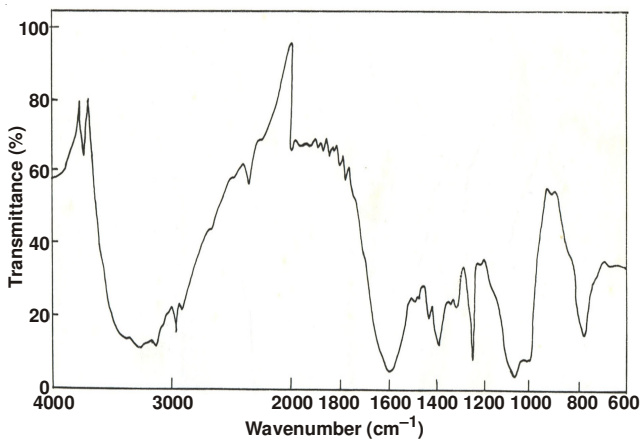

Fig. 5. Infrared spectrum of the copper-zinc complexes

Cyclic voltammetry studies: Since the complexes are insoluble in water, DMF and DMSO, the cyclic voltammetric measurements were carried out in 0.01N HCl using tetraethyl ammonium perchlorate as a supporting electrolyte. The potentials are reported against Ag/AgCl electrode. The complex shows two irreversible peaks at -1.16 and -1.42 V, respectively as shown in Fig. 6. The first peak is due to the reduction of Cu(II) to Cu(I) and the next one is attributed to the reduction of imidazole ring. The small peak observed in the cathodic and anodic portions at -0.5 and -0.2 V, respectively are due to oxygen. The reduction of Cu(II) to Cu(I) and the observation of oxygen peak is in accordance with the mechanism suggested for disproportionation of O_2^- by SOD as shown in the Fig. 7.

Comparison of the suggested mechanism and the cyclic voltammetry behaviour of model complex $[CnZn(his)_3(H_2O)_2]$, it is indicated that Cu(II) undergoes reduction to Cu(I). Thus Cu(II) assists in the conversion of O_2^--O_2^{2-} and to O_2, which is a disproportionation reaction. The oxygen thus generated is seen in cyclic voltammetry in the aqueous solution at -0.5 V as cathodic peak and at -0.2 and -0.11 V as anodic peaks. Thus the complex can act as the model complex for the disproportionation of superoxide ion as it shows the formation of Cu(I) and oxygen in aqueous solution.

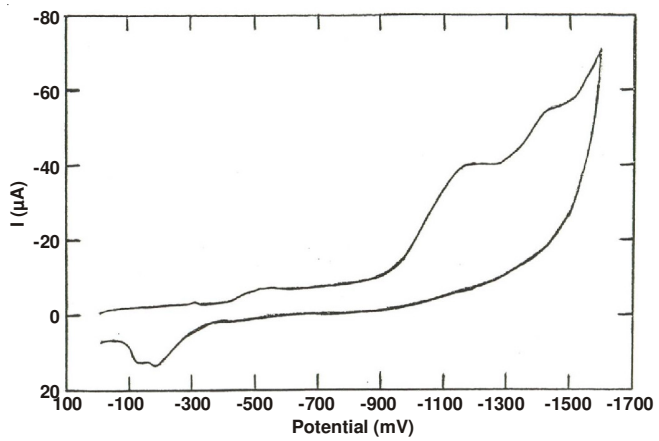

Fig. 6. Cyclic voltammetry of copper-zinc histidine complex

Comparison of $[CuZn(his)_3(H_2O)_2]$ with Cu-Zn-SOD: The native enzyme copper-zinc superoxide dismutase disproportionates the superoxide anions into hydrogen peroxide and water. This enzyme contains two metal ions: copper(II) and zinc(II). Copper(II) gives the blue colour to the enzyme and shows a λ_{max} 680 nm (14,700 cm^{-1}) and also shows shoulder at 417 nm (14,700 cm^{-1}) which is believed due to be an imidazolate-to-copper charge transfer transition[17]. But the synthesized $[CuZn(his)_3(H_2O)_2]$ complex in aqueous solution showed λ_{max} at 710 nm. The shift in λ_{max} of the complex can be the difference in ligand field strength of histidine in the enzyme moiety and in discrete complex environment. The λ_{max} suggests an asymmetric ligand arrangement around the Cu(II) metal ion by nitrogen atoms of histidine and water molecules as showed in the case of native enzyme SOD.

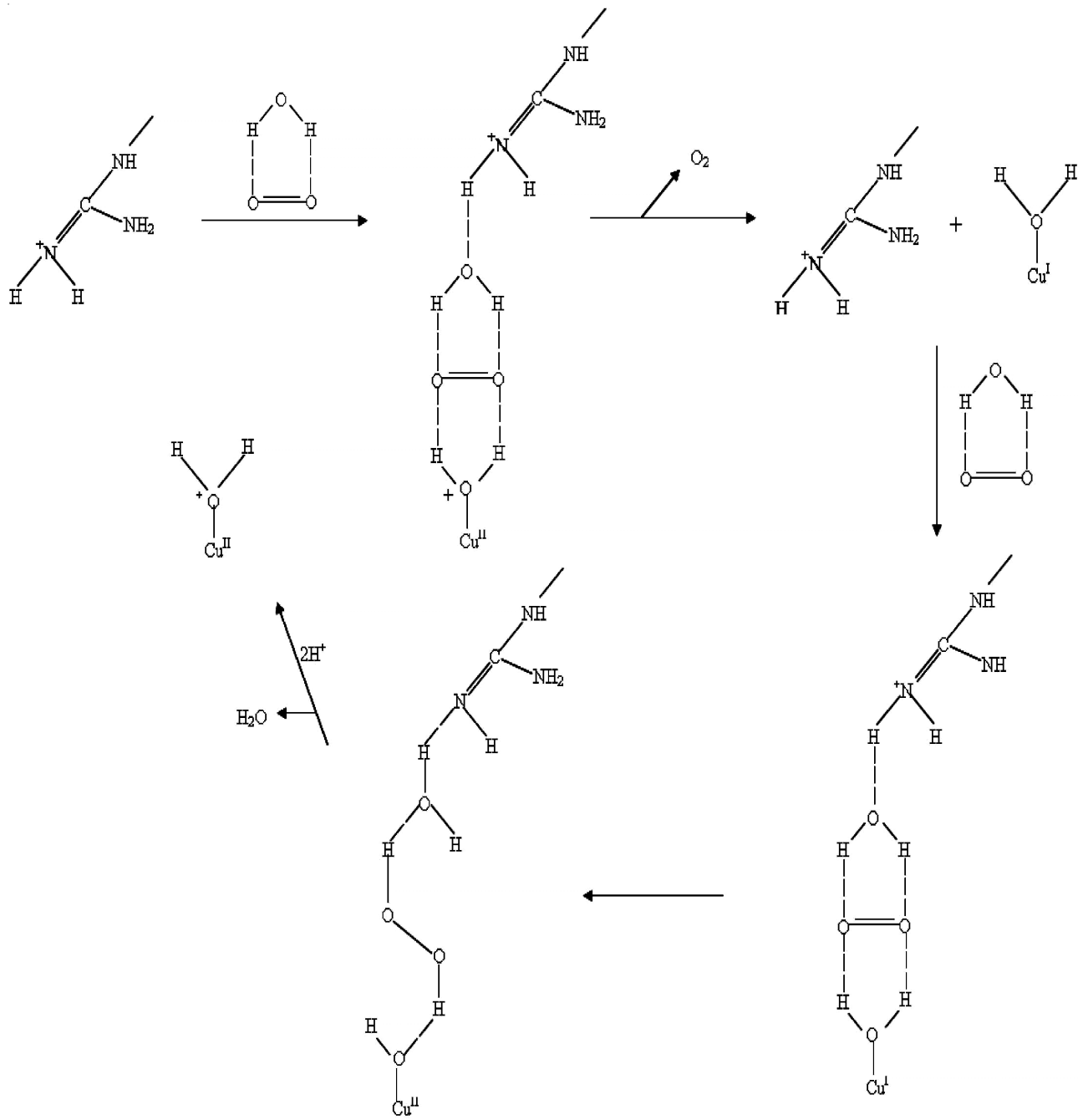

Fig. 7. Mechanism for disproportionation of O^{2-} by SOD

Zinc(II) is a d^{10} metal ion which is inactive in EPR spectra, but provide stability to the complex by coordinating through the bridging histidine molecule. From the analytical data and physical evidences the stoichiometry of the complex $[CnZn(his)_3(H_2O)_2]$ can be proposed by the following equation.

$$2[Cu(his)_2] + 2Zn(his)_2{\cdot}2H_2O \rightarrow 2[CuZn(his)_3(H_2O)_2] + (his)_2{\cdot}2H_2O$$

The complex can be represented as a dimer corresponding to the formula $[CnZn(his)_3(H_2O)_2]$ as the native enzyme Cu-Zn-SOD, which is existing as a dimer. The proposed structure for the monomer of the complex is given Fig. 8.

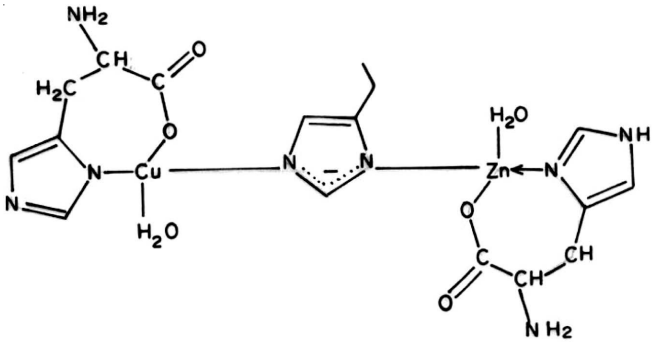

Fig. 8. Proposed structure of copper-zinc heterobinuclear complex

The native enzyme showed similar type of imidazolate anion bridging. In the enzyme Cu-Zn-SOD the copper(II) and zinc(II) metal ions share a common ligand, the imidazolate ring of His 61, which is in the form of the deprotonated imidazolate anion and bridges the coppeer and zinc ions, holding them at a distance of 6.3 Å apart. The deprotonation of the -NH group in the imidazole part of the histidine has been suggested in accordance with similar deprotonation and coordination was infered, in the native Cu-Zn-SOD and in the related model complexes. Histidine acts a bivalent bidentate ligand to copper, where one of the histidine moieties acts as a bridging ligand exactly as proposed in the native Cu-Zn-SOD. The divalent nature of copper(II) and zinc(II) could only be explained in the present complex considering the imidazolate anion as bridging.

Decomposition studies of hydrogen peroxide: The catalytic decomposition of hydrogen peroxide with $[CuZn(gly)_4(H_2O)_2]$ complex and $[CuZn(his)_3(H_2O)_2]$ complex were studied using 0.1 N potassium permanganate at room temperature to distinguish the synthetic complexes and mechanical mixtures. Equal volumes of hydrogen peroxide solution were added to each of the complex solution. A constant volume of these mixtures were titrated against standardized $KMnO_4$ at every 10 min interval were plotted as shown in Fig. 9, which shows that the complex $[Cu(gly)_2]$ reacts rapidly with hydrogen peroxide as indicated by the curve d, whereas $[Zn(gly)_2]$ reacts at lower rate as shown by curve a of Fig. 9(A). The curves of b and c represents the decomposition nature of $[CuZn(gly)_4(H_2O)_2]$ complex and its mechanical mixture respectively and these curves behave similarly, showing that the decomposition of hydrogen peroxide takes place at similar rate. Since, the titration values were very similar and the curves were same

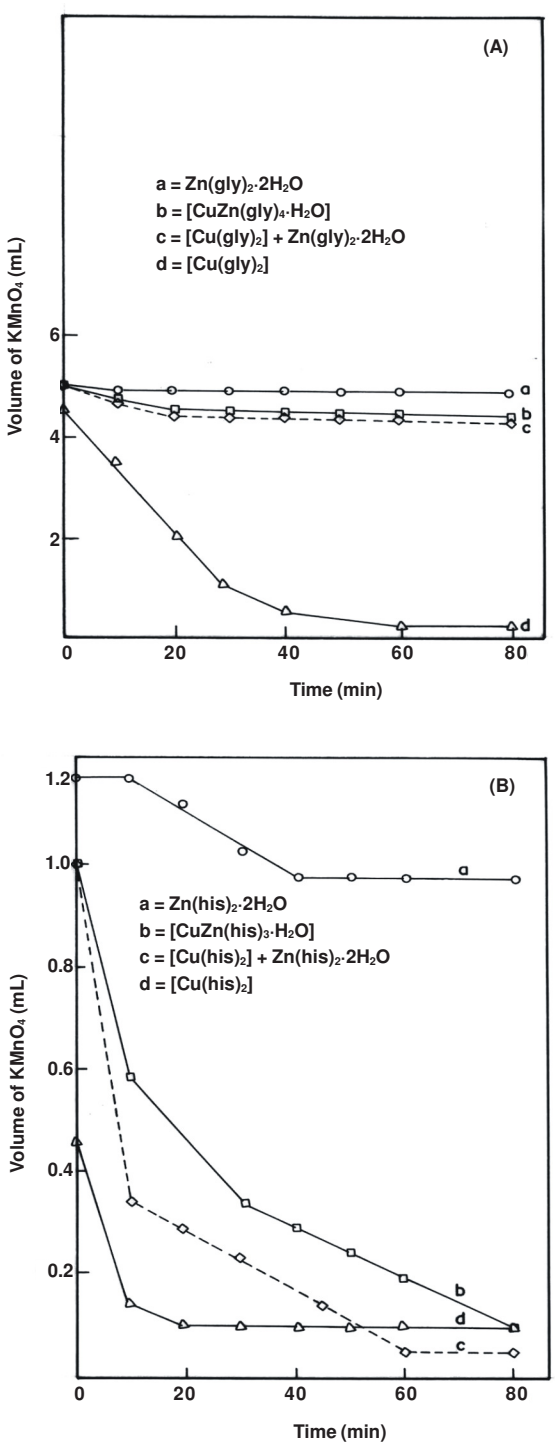

Fig. 9. (A) Plot of decomposition of hydrogen peroxide for glycine complex (B) plot of decomposition of hydrogen peroxide for histidine complexes

showing no chemical interaction between the two metal complexes (Fig. 9(A)). A similar study was done for histidine system where $[Zn(his)_2]$ reacts slowly and $[Cu(his)_2]$ reacts rapidly as indicated by curves a and d, respectively of Fig. 9(B). The curve b and c of Fig. 9(B), represents the decomposition nature of $[CuZn(his)_3(H_2O)_2]$ complex and its mechanical mixture respectively. Here the volume of $KMnO_4$ *versus* time curves of the mechanical mixture was completely different. The difference in pattern of these curves indicates that the

discrete complex was radically different showing the formation of a binuclear complex.

Conclusion

The hetero-binuclear complex of copper(II) and zinc(II) metal ions with the chosen amino acids glycine and histidine were prepared in benzene medium. The characterizations of these compounds were carried out by conductance measurements, electronic, EPR, infrared spectral studies and cyclic voltammetry. The results of the above physical methods show that the histidine favours the synthesis of hetero-binuclear complex with copper(II) and zinc(II) metal ions whereas glycine does not favour such a complex. The special coordination behaviour of histidine is its ability to act as a bridging ligand between the two metal ions, through the nitrogen atoms of imidazolate anion. The optical spectrum of the native enzyme and the isolated complex $[CuZn(his)_3(H_2O)_2]$ are similar in behaviour. The catalytic decomposition of hydrogen peroxide study supports the above results. Thus the synthetic complex $[CuZn(his)_3(H_2O)_2]$ can mimic the enzyme Cu-Zn-SOD structurally and spectroscopically.

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