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Effects of imidazole and zinc on the interaction of some amino acid compounds of copper(II) with hydrogen peroxide

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A novel effect of the inhibition of the decomposition of amino acids to carbonates on addition of imidazole (HIm) to a reacting system containing equimolar amounts of copper and zinc metal powders, an amino acid [glycine (Hgly), aspartic acid (H_2Asp) or glycylglycine (H_2gg)] (1:1:2) and excess hydrogen peroxide (H_2O_2) resulting in formation of a mixed metal mixed ligand peroxo complex compound was observed, because in the absence of imidazole the corresponding reaction system yields only a mixed metal peroxo carbonate. For the resulting complex compounds, the homogeneity, i.e. $[Cu(Zn)(O_2^{2-})(Gly)_2(HIm)(H_2O)]$, $[Cu(Zn)(O_2^{2-})(Asp)(HIm)(H_2O)_2]$ or $[Cu(Zn)_2(O_2^{2-})_2(gg)(HIm)(H_2O)_4]$, molecular formula, presence of peroxo group and coordination environment were established by combined physicochemical evidence from elemental and thermogravimetric analysis in air and argon atmospheres, electron spin resonance and electronic and IR spectral data. It is noteworthy to mention that the corresponding carboxylic acids of the above-mentioned amino acids, i.e. acetic and succinic acids, either do not decompose to carbonates in the absence of imidazole or form novel homogeneous peroxo mixed metal mixed ligand complex compounds as described above in the presence of imidazole. This suggests an important and significant mutual influence (*in vitro*) of biologically active chromophores like peroxo ions, imidazole and amino groups in the above-mentioned chemical reactions containing bioactive metals such as copper and zinc.

Keywords: amino acids, copper, glycylglycine, hydrogen peroxide, imidazole, zinc

Introduction

A large number of redox reactions occur in biological systems which are catalyzed by enzymes containing copper, zinc, etc., as metal centers (Solomon *et al.* 1983). Hydrogen peroxide (H_2O_2) is a metabolic byproduct in many living systems. Copper(II) is one of the important transition metal ions found in redox enzymes like superoxide dismutase (SOD), which decomposes harmful superoxide ion radicals present in all aerobic organisms (including humans) to dioxygen and H_2O_2 (Solomon *et al.* 1983, Pate *et al.* 1987). The active site of bovine erythrocyte SOD enzyme has Zn(II) and Cu(II) ions bridged by an imidazolate group and surrounded by an imidazole moiety of a histidine residue and aspartate ion (Tainer *et al.* 1983, Sato *et al.* 1984). In view of the importance of this enzyme, many model compounds of Cu(II) mimicking the coordination site of the enzyme have been reported (Bell *et al.* 1969, Sato *et al.* 1984). In addition to the formation of simple peroxo and bridging peroxo complexes containing various bonding modes, H_2O_2 is also capable of liberating powerful oxidative free radical species like $OH^{\cdot}$, O_2^{-} etc., and dioxygen during its reactions with various substrates. However, very little is known about the influence of either the ligands (like imidazole) or metal ions (like zinc) on the interaction of simple copper amino acid compounds with H_2O_2. The study of such systems would be of interest among other things to understand the mutual influence of ligands and metal ions on the complex species present in biological fluids. Recently we have observed a profound effect of zinc metal on the interaction of Cu(II) amino acid compounds with H_2O_2, i.e. decomposition of the amino acids to carbonates by a deamination process (while with corresponding simple Cu(II) carboxylate systems no such effect was observed) with the formation of a novel mixed metal peroxo carbonate (Sastry *et al.* 1992). In view of these interesting aspects, the objectives of this work was to study the mutual influence of various ligand and metal ions

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on the above system by isolating the reaction products and characterizing them by various physicochemical methods. This paper reports the novel effect of the addition of imidazole to a reaction system containing amino acids, i.e. glycine, aspartic acid and glycylglycine, H_2O_2 and bioactive metals, i.e. copper and zinc, by isolating the reaction products and characterizing them by elemental and thermal analysis, electron spin resonance (ESR), and electronic and IR spectroscopy techniques.

Materials and methods

All chemicals and reagents used were of AR grade. H_2O_2 (30% aqueous) solution was used for reactions. The reactions were carried out in aqueous media at ordinary atmospheric pressure.

IR spectra were recorded with an FTIR cygnus 100 spectrometer using CsI discs with both nujol and flurolub mulls. Diffuse reflectance spectra of solid complexes were taken on a Shimadzu model 210-A spectrophotometer using $BaSO_4$ as a reference standard in the region 200–800 nm. ESR spectra of polycrystalline compounds were recorded both at room and liquid nitrogen temperatures at X-band frequencies on a Bruker ESP 300 spectrometer using DPPH as an internal field marker. Thermogravimetric analyses (TGA) were carried out in air on a Shimadzu DT-30 micro TG instrument, with the sample weight in the 10 mg range.

Copper and zinc were determined by the atomic absorption method. Carbon, hydrogen and nitrogen were determined by microanalysis. Peroxo oxygen atoms (O_2^{2-}) were determined by the ceric sulfate method (Vogel 1964).

Preparation of [Cu(Zn)(O_2^{2-})(Gly)$_2$(HIm)(H_2O)] (1)

Compound **1** was obtained as a light green precipitate along with a clear colorless supernatant liquid by constant stirring for 5 days of an aqueous reaction mixture containing copper and zinc metal powders, glycine and imidazole (1:1:2:4, respectively) in excess H_2O_2 (about 3 ml). Initially, copper powder started reacting, giving a pale blue solution, and the reaction was carried at room temperature for 3 days whereby all the copper dissolved and then heated to about 50 °C (on a water-bath) with periodical addition of 1 ml H_2O_2 solution for 2 days to facilitate the completion of the reaction. Gradually, all of the zinc powder started dissolving to give a green precipitate with a simultaneous corresponding decrease in the intensity of supernatant dark blue color, along with some evolution of ammonia gas (identified by Necessler's reagent test, smell and turning of moist pH paper to a blueish green at the mouth of the reaction vessel). The green precipitate was washed thoroughly with water and acetone and dried in vacuum.

Analysis. Calculated for $CuZnC_7N_4H_{14}O_7$: Cu, 16.09; Zn, 16.55, C, 21.27; N, 14.18; H, 3.54; O_2^{2-}, 8.1. Found: Cu, 15.87; Zn, 15.95; C, 20.95; N, 14.5; H, 3.14; O_2^{2-}, 8.45%.

Preparation of [Cu(Zn)(O_2^{2-})(Asp)(HIm)(H_2O)$_2$] (2)

The preparation of compound **2** (green) was analogous to **1**.

Analysis. Calculated for $CuZnC_7N_3H_{13}O_8$: Cu, 16.04; Zn, 16.50, C, 21.21; N, 10.60; H, 3.28; O_2^{2-}, 8.08. Found: Cu, 15.93; Zn, 16.10; C, 21.0; N, 10.70; H, 2.95; O_2^{2-}, 7.5%.

Preparation of [Cu(Zn)$_2$(O_2^{2-})$_2$(gg)(HIm)(H_2O)$_4$] (3)

The isolation and preparation of **3** (light green) was analogous to **1** and **2** except that the supernatant liquid showed a light blue color.

Analysis. Calculated for $Cu(Zn)_2C_7N_4H_{18}O_{11}$: Cu, 12.02; Zn, 24.74, C, 15.90; N, 10.60; H, 3.40; O_2^{2-}, 12.10. Found: Cu, 12.02; Zn, 24.46; C, 15.5; N, 10.5; H, 3.10; O_2^{2-}, 11.57%.

Solubilities

All compounds (**1–3**) were stable at room temperature and were insoluble in common organic solvents. Their solubility in water was very low and they decomposed without melting beyond 260 °C.

Results and discussion

ESR spectra of powdered compounds (**1–3**) were taken both at 298 and 77 K. Compounds **1** and **2** exhibit an isotropic spectrum at both temperatures with g_{iso} values of 2.050 and 2.077, respectively, while **3** shows an axial spectrum at both these temperatures (Figure 1) with $g_{11} = 2.126$ and $g_{\perp} = 2.095$ ($g_{av} = 2.105$). The nature of the ESR spectra and the g values of compounds **1–3** clearly rule out these complex compounds being mixtures of their individual components. This is supported by comparing the nature of the ESR spectra and g values reported for the corresponding individual Cu(II) and mixed metal Cu(II)–Zn(II) complexes, i.e. copper peroxy glycine, copper peroxy aspartate, copper peroxy glycylglycine copper and copper–zinc peroxy imidazole (Sastry *et al.* 1992, 1994). Also, the reported non-peroxo compound of copper aspartate imidazole (Antolini *et al.* 1982) has an axial spectrum in contrast to the isotropic spectrum with a different g value for Cu(II) in the corresponding peroxo compound **2** and the corresponding non-peroxo compound **3**, [Cu(gg)(HIm)]2H_2O and/or [Cu(gg)(H_2O)] have different g and λ_{max} values (Nakao *et al.* 1981), thus corroborating the different set of coordination groups and geometry around Cu(II) in **2** and **3**.

The nature of the electronic absorption (reflectance) spectra of **1–3** are similar, with a broad peak due to the d–d electronic transition of Cu(II) at 650, 660 and 675 nm, respectively, along with an intense broad band around 350 nm (Figure 2). The variation of λ_{max} values for **1–3** is in accordance with the presence of different ligand environments around the Cu(II) ion in these compounds.

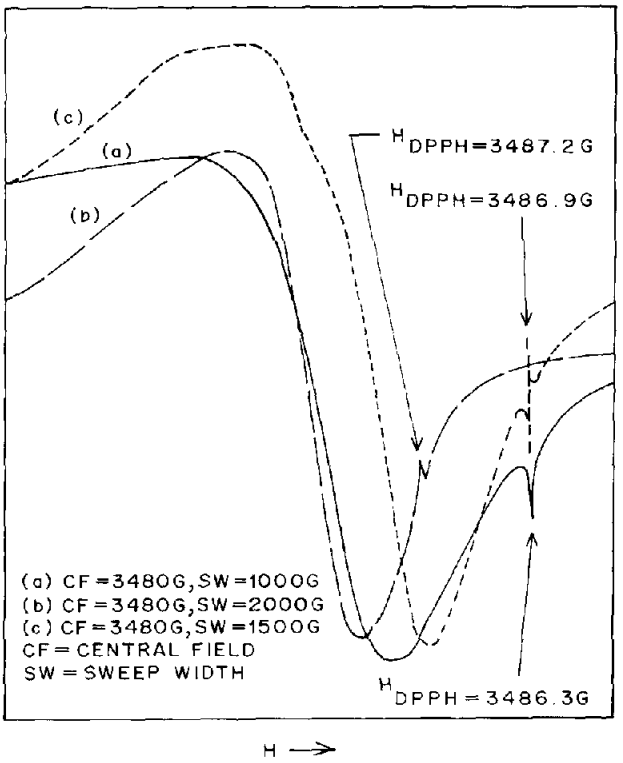

Figure 1. ESR spectrum at 298 K recorded with 5 G modulation amplitude, 2 mW power and frequency 9.74 GHz.
(**a**) $[Cu(Zn)(O_2^{2-})(Gly)_2(HIm)(H_2O)]$,
(**b**) $[Cu(Zn)(O_2^{2-})(Asp)(HIm)(H_2O)_2]$ and
(**c**) $[Cu(Zn)_2(O_2^{2-})_2(gg)(HIm)(H_2O)_4]$.

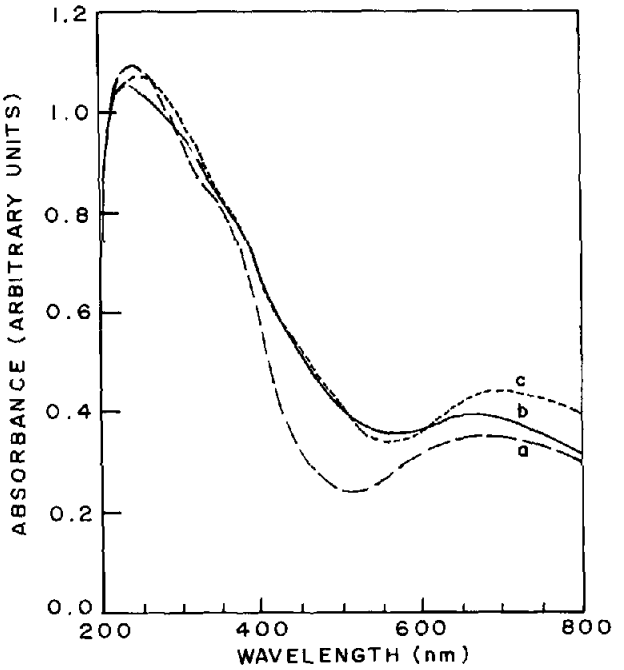

Figure 2. Electronic (reflectance) spectra of
(**a**) $[Cu(Zn)(O_2^{2-})(Gly)_2(HIm)(H_2O)]$,
(**b**) $[Cu(Zn)(O_2^{2-})(Asp)(HIm)(H_2O)_2]$ and
(**c**) $[Cu(Zn)_2(O_2^{2-})_2(gg)(HIm)(H_2O)_4]$.

Also, in **3** the shift to the lower energy of the Cu(II) *d–d* band (675 nm) to the band observed at 613 nm for the corresponding non-peroxo compound (Driver & Walker 1968) clearly corroborates the presence of a different set of coordination atoms. The red shift of the *d–d* band ($\Delta\lambda_{max} \approx 62$ nm) is in accordance with the replacement of the coordinated ligand nitrogen atoms of the corresponding non-peroxo complex of **3** by a divalent peroxo (O_2^{2-}) group of compound **3** (Mori *et al.* 1979, Thompson 1984).

The complexity of structures and overlap of imidazole and amino acid IR absorption peaks in the regions of interest for structural characterization of **1–3** precludes any unambiguous assignments. The IR spectra of **1–3** exhibits two strong broad bands (3365 and 3152, 3334 and 3147, and 3320 and 3155 cm^{-1} for **1–3**, respectively) in the 3000–3400 cm^{-1} region in addition to strong water absorption bands around 3400 cm^{-1}. This suggests coordination of imidazole as a neutral molecule and amino acid as a deprotonated chelating ligand in **1–3**, because if imidazole acts as a deprotonated imidazolate ion and amino acid as a neutral zwitterion molecule then only one band (either due to the absence of the NH or imidazole or the presence of NH_3^+ of amino acid) is expected in that region (Bauman & Wang 1964, Nakao *et al.* 1981). Although the observed IR data of **1–3** are not helpful in the unambiguous identification of the amino acid either as a neutral molecule or a deprotonated acid, the stoichiometric analysis and valency considerations of the metal ions suggest the deprotonation of the amino acid. Accordingly, the bands observed at 1700, 1650, 1618 (compound **1**), 1702, 1659, 1613 (compound **2**), 1700, 1655, 1615 (compound **3**) are tentatively attributed to the NH deformation mode of neutral imidazole and to the coordinated COO^- group of the amino acid respectively (Bauman & Wang 1964, Nakao *et al.* 1981, Antolini *et al.* 1982). In **1–3**, medium bands at 800, 789 and 795 cm^{-1}, respectively, are assigned to $\nu(O_2^{2-})$ based on the absence of IR absorption bands in this region for the corresponding non-peroxo complexes (Mori *et al.* 1979, Nakao *et al.* 1981, Antolini *et al.* 1982) and in accordance with the reported assignments for the related compounds (Sastry *et al.* 1992). The presence of water molecules is identified from the ν(OH) bands at 3395, 3375 and 3335 cm^{-1} for **1–3**, respectively. The appearance of ν(OH) vibrations below 3400 cm^{-1} and the temperature (> 150 °C) at which the initial weight loss occurs due to water molecules observed from TGA curves (Figure 3) corroborates the presence of coordinated water instead of lattice water (Sastry *et al.* 1992).

TGA of compounds **1–3** were carried out in air from 27 to 950 °C (Figure 3). The residue in each case (end product) was identified as a mixture of copper oxide and zinc oxide by X-ray diffraction studies. The close agreement of the expected and observed weight loss both for the number of water molecules in the 100–200 °C range and total weight loss from the analysis of TG curves (Figure 3) clearly supports the molecular formula, thus corroborating

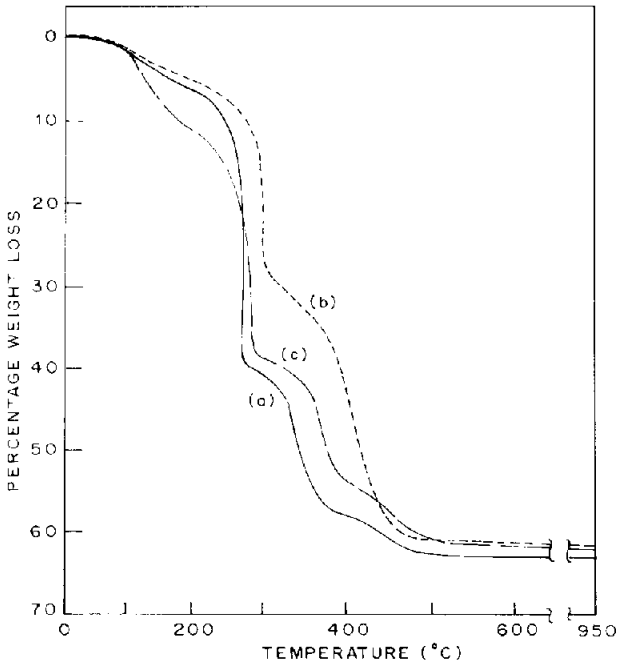

Figure 3. TG curve in air (heating rate 10 °C min^{-1}; chart speed 2.5 mm min^{-1}). (**a**) $[Cu(Zn)(O_2^{2-})(Gly)_2(HIm)(H_2O)]$. (**b**) $[Cu(Zn)(O_2^{2-})(Asp)(HIm)(H_2O)_2]$ and (**c**) $[Cu(Zn)_2(O_2^{2-})_2(gg)(HIm)(H_2O)_4]$.

the stoichiometric formulae arrived at from elemental analysis and the estimation of peroxo oxygen.

Thus the combined evidence of elemental and thermogravimetric analysis, ESR, and electronic and IR data clearly indicates that compounds **1–3** are homogeneous (not a mixture of individual components) containing coordinated imidazole, amino acid, peroxo and water molecules in their coordination sphere. It is also of interest to mention that while the corresponding non-peroxo compound of **1** has not been reported, the corresponding non-peroxo compounds for **2** and **3** have been well characterized (Nakao *et al.* 1981). Hence these compounds are structurally formulated as: $[Cu(Zn)(O_2^{2-})(Gly)_2(HIm)(H_2O)]$, $[Cu(Zn)(O_2^{2-})(Asp)_2(HIm)(H_2O)_2]$ and $[Cu(Zn)_2(O_2^{2-})_2(gg)(HIm)(H_2O)_4]$.

Perhaps the most noticeable variation in the above series of compounds is the difference in the stoichiometric ratio of copper and zinc in **3**, and, then, the gradual change in the number of water molecules in **1–3**, both of which were corroborated from elemental and thermogravimetric analyses. It is to be pointed out here that the composition of **1–3** indicates only 1 mol of imidazole, whereas 4 mol of imidazole was taken for reaction in all three systems. It was also observed that the filtrate after isolating the reaction product did not give any solid on evaporation. Hence it is interesting to find that the excess imidazole in all cases was decomposed to ammonia, which was observed during the course of the reaction. It is also important to mention that the reactions involving the corresponding simple carboxylic acids and imidazole did not yield any ammonia gas or form any homogeneous reaction products, suggesting the partial decomposition of imidazole to ammonia occurs only in the presence of amino acids.

It is likely that the imidazole added to the above-mentioned reaction mixtures removes Cu(II) and Zn(II) metal centers as imidazole complex species, which in turn react with amino acid molecules resulting in the formation of **1–3**, because in the absence of imidazole the reaction mixture results in decomposition of amino acids to carbonate. Also it is pertinent to note that equimolar mixtures of copper and zinc powders with amino acids and imidazole in the absence of H_2O_2 did not react even on heating in a water bath and stirring for a few days; and the reaction of copper powder with amino acids and imidazole in the presence of H_2O_2 yielded a mixture of different colored compounds on evaporating the solution at room temperature over a desiccator. This suggests unequivocally the mutual influence of reactants on the formation of compounds **1–3** and on the inhibition of the decomposition of copper amino acid chelates to carbonates.

To conclude, the preceding discussion clearly indicated an interesting and perhaps significant effect of coordination of peroxo ions, amino acids and imidazole groups to copper and zinc ions in the formation of novel mixed metal mixed ligand peroxo complexes. This is suggestive of a mutual influence of these biologically active chromophores in some important biological reactions (*in vitro*).

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