Synthesis, characterization and superoxide dismutase activity of some ternary copper(II) dipeptide–2,2′-bipyridine, 1,10-phenanthroline and 2,9-dimethyl-1,10-phenanthroline complexes

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Abstract

Several complexes of 2,2′-bipyridine, 1,10-phenanthroline and 2,9-dimethyl-1,10-phenanthroline with copper(II) dipeptides have been synthesized. These complexes are neutral and show a broad absorption band in the range 630–640 nm due to d–d transition. The solution and glassy state ESR spectra of the complexes have been measured at 301 and 77 K, respectively. The magnetic and bonding parameters of these complexes have been calculated from the above data. The ESR spectral data suggest that these complexes have distorted square pyramidal geometry about Cu(II). The superoxide dismutase activity of several of the above complexes has been measured. They show higher activity than the corresponding Cu(II) dipeptide complexes because of a strong axial bond of one of the nitrogen atoms of the α-diimine.

Introduction

Copper(II) complexes have found possible medical uses in the treatment of many diseases including cancer [1, 2]. It has been suggested that the anticancer activity of some copper(II) complexes may be based on their ability to inhibit DNA synthesis [1]. One further possible mechanism of action that has been studied for certain complexes involves the scavengering of superoxide anions [1]. Cu(II)-3,4-diisopropyl-salicylate [3], Cu(II)(glycylglycylhistidine) [4], Cu(II)(glycylhistidyllysine) [5], Cu(II)(pyridine-2-carboxaldehyde-2-pyridylhydrazone), Cu(II)(salicylaldehydehydrazone) [6], Cu(II) bleomycin, Cu(II) thiosemicarbazones [7], Cu(II) amino acid complexes [8], Cu(II) complexes of 2,9-dimethyl-1,10-phenanthroline and 3,4,7,8-tetramethyl-1,10-phenanthroline [9, 10], *trans*-bis(salicylaldoximato)Cu(II) [11], Cu(II) complex of tetrabenzo[*b*,*f*,*j*,*n*]-1,5,9,13-tetraazacyclohexadecane [12], Cu(II) complexes of trihydroxybenzohydroxamic acid [13] and bis-(acetato)bis(imidazole)Cu(II) [14] have been reported to be effective in Elrich cell ascites tumor or similar animal tumor models as well as in tumor cell cultures. 2,2′-Bipyridine and 1,10-phenanthroline chelators also act as potential antitumor agents [15, 16]. They can have even better antitumor activity if their hydrophilic groups are masked by copper ions to form water soluble neutral chelates. These neutral complexes are expected to be more permeable through the cell membrane [17, 18]. In this paper, we report the synthesis, characterization and superoxide dismutase activity of some neutral ternary complexes of copper(II) dipeptides with 2,2′-bipyridine, 1,10-phenanthroline and 2,9-dimethyl-1,10-phenanthroline (neocuproine).

Experimental

Glycylglycine (Gly·Gly), glycyl-L-alanine (Gly·Ala), glycyl-L-phenylalanine (Gly·Phe), glycyl-L-tyrosine (Gly·Tyr), and bovine erythrocyte superoxide dismutase were bought from Sigma, U.S.A. 2,2′-Bipyridine (bipy), 1,10-phenanthroline (phen), 2,9-dimethyl-1,10-phenanthroline (dmph) and nitro blue tetrazolium chloride (NBT) were purchased from SRL, India. Other chemicals were analytical reagent grade and were used as such. The solvents used were purified before use by the standard method [19].

The absorption and electron spin resonance (ESR) spectra were recorded as described earlier [20]. The Monte Carlo computer simulation program was used

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to determine accurate ESR parameters of copper(II) complexes at 77 K [21]. Microanalysis for C, H and N in the Cu(II) complexes was carried out at the Microanalytical Laboratory, IIT Bombay.

Cu(Gly·Gly)·3H$_2$O, Cu(Gly·Ala)·2H$_2$O and Cu(Gly·Tyr)·4H$_2$O

These complexes were prepared as described elsewhere [22–24].

Cu(Gly·Gly)(bipy)·3H$_2$O, Cu(Gly·Gly)(phen)·3H$_2$O, Cu(Gly·Phe)(bipy)·4H$_2$O, Cu(Gly·Tyr)-(bipy)·4H$_2$O and Cu(Gly·Tyr)(phen)·3H$_2$O

These complexes were synthesized as given earlier [25].

All of these complexes gave satisfactory chemical analyses.

Cu(Gly·Ala)(bipy)·4H$_2$O

Cu(Gly·Ala)·2H$_2$O (1 mmol) was dissolved in 30 ml of aqueous methanol. A solution of 2,2′-bipyridine (1 mmol) in 25 ml of methanol was added to it. The mixture was stirred for 1 h and then filtered. The bluish green filtrate was concentrated to a small volume at 28 °C. The blue crystals were collected by filtration, recrystallized from methanol and air dried. *Anal.* Calc. for $C_{15}H_{24}N_4O_7Cu$: C, 41.33; H, 5.51; N, 12.90. Found: C, 41.20; H, 5.50; N, 12.50%.

Cu(Gly·Ala)(phen)·3H$_2$O

This complex was synthesized by following the preparative method of Cu(Gly·Ala)(bipy)·4H$_2$O, except that phen was used in place of bipy. *Anal.* Calc. for $C_{17}H_{22}N_4O_6Cu$: C, 46.21; H, 4.97; N, 12.70. Found: C, 46.20, H, 5.00; N, 12.80%.

Cu(Gly·Phe)(phen)·3H$_2$O

This complex was synthesized by following the preparative method of Cu(Gly·Phe)(bipy)·4H$_2$O [24] except that phen was used in place of bipy. *Anal.* Calc. for $C_{23}H_{26}N_4O_6Cu$: C, 53.33; H, 5.02; N, 10.82. Found: C, 53.50, H, 4.90; N, 10.30%.

Cu(Gly·Gly)(dmph)·4H$_2$O

This complex was prepared as Cu(Gly·Ala)-(bipy)·4H$_2$O except that Cu(Gly·Gly)·3H$_2$O and dmph were used in place of Cu(Gly·Ala)·2H$_2$O and bipy, respectively. *Anal.* Calc. for $C_{18}H_{26}N_4O_7Cu$: C, 45.62; H, 5.49; N, 11.85. Found: C, 46.00, H, 5.30; N, 11.83%.

Cu(Gly·Tyr)(dmph)·4H$_2$O

This complex was prepared in the same way as Cu(Gly·Gly)(dmph)·4H$_2$O except that Cu(Gly·Tyr)·4H$_2$O was used in place of Cu(Gly·Gly)·3H$_2$O. *Anal.* Calc. for $C_{25}H_{32}N_4O_8Cu$: C, 51.77; H, 5.52; N, 9.66. Found: C, 51.60 H, 5.30; N, 9.80%.

Alkaline dimethyl superoxide–nitro blue tetrazolium assay

The alkaline DMSO–NBT method used here has been discussed earlier [20, 26]. In this method, a typical 400 μl sample to be assayed was added to a solution containing 2.1 ml of 0.2 M potassium phosphate buffer (pH 8.6) and 1 ml of 56 μM NBT. The tubes were kept in ice for 15 min and then 1.5 ml of alkaline DMSO solution containing superoxide ions was added with stirring. The absorbance of the violet colour developed was monitored at 560 nm against a sample prepared under similar conditions except that NaOH was absent in DMSO. A unit superoxide dismutase (SOD) activity is the concentration of complex or enzyme which causes 50% inhibition of alkaline dimethyl superoxide (DMSO) mediated reduction of nitro blue tetrazolium chloride (NBT).

Results and discussion

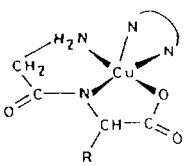
The molar conductance values and electronic absorption maxima of ternary α-diimine adducts of copper(II) dipeptides are given in Table 1. These neutral complexes show only one broad band in the range 630–640 nm. The α-diimine adducts of Cu(II) glycyl–L-alanine show lower λ_{max} values than corresponding α-diimine adducts of Cu(II)glycyl–L-phenylalanine. In the α-diimine adducts of Cu(II) dipeptides, the value of λ_{max} increases in the following order: Cu(II) glycyl–L-alanine adducts < Cu(II) glycyl–L-phenylalanine adducts < Cu(II) glycyl–L-tyrosine adducts < Cu(II) glycylglycine adducts. 2,9-Dimethyl-1,10-phenanthroline adducts have fairly low λ_{max} as compared to the corresponding 2,2′-bipyridine and 1,10-phenanthroline adducts. The molar absorption coefficients of these adducts are in the range 90–135, which are higher than that of simple Cu(II) dipeptides [25] indicating distortion from D_{4h} symmetry. These adducts show also some absorption at 850 nm. The percentage ratio of ϵ_{850} to ϵ_{max}, which is represented by n is also given in Table 1. The ternary adducts of 2,2′-bipyridine, 1,10-phenanthroline and 2,9-dimethyl-1,10-phenanthroline show values of n ranging from 26 to 45, which is indicative of distortion from D_{4h} symmetry with the stronger axial field around the copper(II) [25, 27]. The crystal structure analysis of 1,10-phenanthroline adducts of copper(II) glycylglycine shows that the glycylglycine dianion coordinates with three binding sites, including the carboxylate group, in a square planar arrangement

TABLE 1. Electronic absorption maxima, molar conductance and SOD activity of copper(II) dipeptide complexes with 2,2'-bipyridine, 1,10-phenanthroline and 2,9-dimethyl-1,10-phenanthroline

Complex	λ_{max} (nm) (ϵ_{max})[a]	n	Molar conductance (ohm^{-1} cm^2 mol^{-1})	Concentration required for unit SOD activity (μM)[b]
Cu(Gly·Ala)(bipy)·$4H_2O$ (a)	633 (109)	37	21	55
Cu(Gly·Ala)(phen)·$3H_2O$ (b)	633 (116)	35	13	35
Cu(Gly·Phe)(bipy)·$4H_2O$ (c)	635 (85)	33	16	30
Cu(Gly·Phe)(phen)·$3H_2O$ (d)	636 (141)	45	18	21
Cu(Gly·Tyr)(bipy)·$4H_2O$ (e)	638 (100)	33	16	16
Cu(Gly·Tyr)(phen)·$3H_2O$ (f)	638 (90)	27	18	13
Cu(Gly·Tyr)(dmph)·$4H_2O$ (g)	630 (124)	30	15	
Cu(Gly·Gly)(bipy)·$3H_2O$ (h)	644 (113)	45	22	25
Cu(Gly·Gly)(phen)·$3H_2O$ (i)	640 (102)	43	26	32
Cu(Gly·Gly)(dmph)·$4H_2O$ (j)	636 (136)	40	20	

[a] ϵ_{max} in parentheses is the molar absorption coefficient at wavelength maximum, λ_{max} (l mol^{-1} cm^{-1}). [b] SOD activity of superoxide dismutase, Cu(Gly·Gly)·$3H_2O$, Cu(Gly·Ala)·$2H_2O$ and Cu(II)(salicylate)$_2$ is 0.72, 132, 800 and 44 μM, respectively by NBT method.

around the copper(II) ion. One of the 1,10-phenanthroline nitrogen occupies the square plane, the other occupies a tilted apical position, giving a distorted square pyramidal geometry around the Cu(II) ion [28–30]. This is consistent with the expected decreasing tendency to occupy the apical position: $Cl^- > COO^-$ (non-peptide) > N (aromatic) > N (amino) [27]. The similarity of the absorption maxima of these α-diimine adducts, their intensity and their infrared spectra further suggest that these complexes are five coordinate with dipeptide ligands behaving as tridentate ligands and α-diimine as bidentate ligands (see Fig. 1).



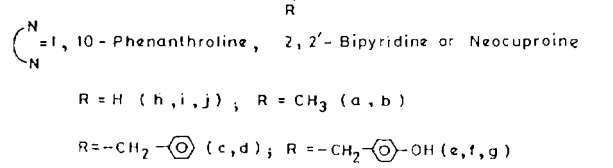


Fig. 1. Structures of ternary complexes of copper(II) dipeptides with α-diimine.

Generally, copper(II) complexes are known to be very labile. Therefore, it may be possible that the ligands already coordinated may be replaced by other ligands. However, these ternary complexes show no change in their electronic absorption spectra in the presence of other ligands in water [25]. This suggests that the dipeptide and 1,10-phenanthroline, 2,2'-bipyridine or 2,9-dimethyl-1,10-phenanthroline are strongly bonded to Cu(II) ion in ternary complexes and the formation of binary or 1:2 (metal:ligand) complexes is less favourable. The stability studies of binary and ternary Cu(II) complexes containing a dipeptide and 1,10-phenanthroline or 2,2'-bipyridine support the above conclusion [28, 31].

The ESR parameters, g and A tensors, for Cu(II) dipeptides and their α-diimine adducts are given in Table 2. A typical frozen solution ESR spectrum is given in Fig. 2.. These α-diimine adducts have lower $g_{\parallel}$ values than those of the corresponding Cu(II) dipeptides. This is due to coordination of one of the nitrogens of 1,10-phenanthroline, 2,2'-bipyridine or 2,9-dimethyl-1,10-phenanthroline, in the equatorial plane. This additional coordination of nitrogen in the equatorial plane introduces a stronger ligand field thereby lowering the $g_{\parallel}$ value [25, 32]. The $g_{\parallel}$ values decrease in these ternary complexes in the

TABLE 2. ESR parameters of copper(II) dipeptide complexes with 2,2'-bipyridine, 1,10-phenanthroline and 2,9-dimethyl-1,10-phenanthroline

Complex	$g_{\parallel}$	$g_{\perp}$	g_0	$A_{\parallel}$[a]	$A_{\perp}$[a]	A_0[a]
Cu(Gly·Ala)(bipy)·$4H_2O$ (a)	2.226	2.078	2.128	164	6	57
Cu(Gly·Ala)(phen)·$3H_2O$ (b)	2.225	2.086	2.132	169	6	58
Cu(Gly·Phe)(bipy)·$4H_2O$ (c)	2.236	2.058	2.118	172	10	61
Cu(Gly·Phe)(phen)·$3H_2O$ (d)	2.231	2.078	2.129	177	19	70
Cu(Gly·Tyr)(bipy)·$4H_2O$ (e)	2.232	2.064	2.120	175	10	62
Cu(Gly·Tyr)(phen)·$3H_2O$ (f)	2.232	2.061	2.118	172	15	65
Cu(Gly·Tyr)(dmph)·$4H_2O$ (g)	2.220	2.085	2.128	171	22	70
Cu(Gly·Gly)(bipy)·$3H_2O$ (h)	2.243	2.058	2.120	157	6	53
Cu(Gly·Gly)(phen)·$3H_2O$ (i)	2.243	2.057	2.119	162	6	55
Cu(Gly·Gly)(dmph)·$4H_2O$ (j)	2.230	2.084	2.133	170	15	65

[a] $(cm^{-1}) \times 10^4$.

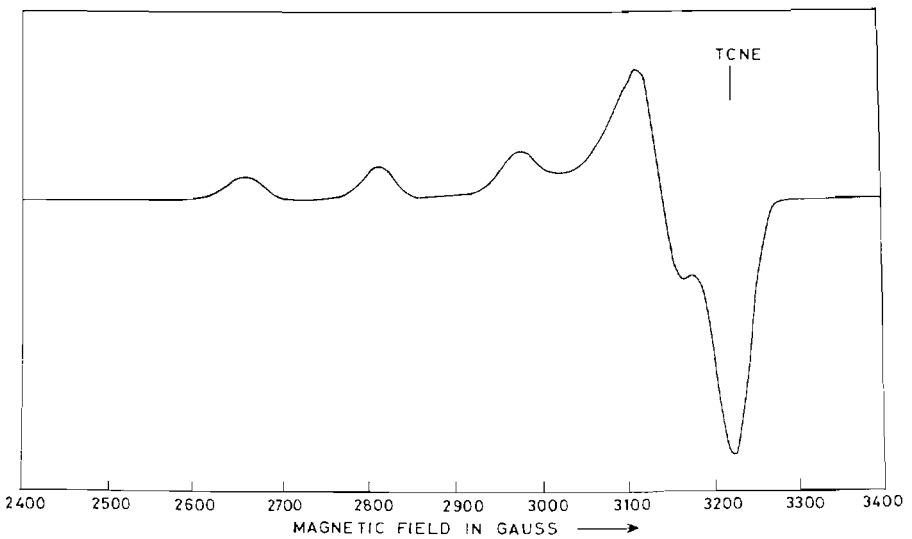


Fig. 2. Frozen aqueous solution ESR spectrum of Cu(Gly·Ala)(Phen)·$3H_2O$ ($\sim 10^{-3}$ M) at 77 K.

order Cu(II) glycylglycine adducts > Cu(II) glycyl–L-tyrosine adducts > Cu(II) glycyl–L-phenylalanine adducts > Cu(II) glycyl–L-alanine adducts. These results are consistent with the blue shifts observed in their absorption spectra. The α-diimine adducts of Cu(II) glycyl–L-alanine have usually lower λ_{max} and $g_{\parallel}$ values than the corresponding α-diimine adducts of other Cu(II) dipeptides. These data indicate a stronger equatorial ligand field in these ternary complexes. The frozen solution ESR spectra of these ternary complexes are typical of square pyramidal geometry with rhombic distortion. A rhombic distortion produces a splitting of the $g_{\perp}$ region into a less intense g_{xx} line on the low field side and a more intense g_{yy} line at higher field [33, 34]. The tendency of rhombic distortion may be measured by the separation between resonances g_{xx} and g_{yy} [35]. This separation in these ternary complexes is between 45 and 50 gauss. The small variations in these values indicate that they possess a rhombically distorted structure which is also shown by the X-ray structural studies of Cu(Gly·Gly)(phen)·$3H_2O$ and Cu(Gly·Gly)-(dmph)·$4H_2O$ [27, 30].

The bonding parameters in the ternary complexes have been estimated using molecular orbital coefficients α, α', β_1 and β obtained by the qualitative method of Kivelson and Neiman [36]. The in-plane σ-bonding is represented by the α^2 values. The totally ionic metal ligand bond gives $\alpha^2 = 1$, while the totally covalent bond gives $\alpha^2 = 0.5$. The α^2 values in these ternary complexes fall between 0.75 and 0.79 (see Table 3). These values are lower as compared to Cu(II) dipeptides. The nitrogen donor in the equatorial plane introduces a stronger ligand field thereby increasing the in-plane covalency in the equatorial plane. This is indicated by a decrease in the α^2 values [37, 38]. The α^2 values for the imidazole adducts are also in the same range, as these complexes also contain three equatorial nitrogen atoms [39].

TABLE 3. Ligand field energies (ΔE^*) bonding parameters and ϵ'^2 values of copper(II) dipeptide complexes with 2,2'-bipyridine, 1,10-phenanthroline and 2,9-dimethyl-1,10-phenanthroline

Complex	ΔE^* (λ_{max}) (cm^{-1})	α^2	α'^2	β_1^2	β^2	ϵ'^2
Cu(Gly·Ala)(bipy)·$4H_2O$ (a)	15797	0.75	0.35	0.82	0.99	0.32
Cu(Gly·Ala)(phen)·$3H_2O$ (b)	15797	0.76	0.33	0.80	0.99	0.32
Cu(Gly·Phe)(bipy)·$4H_2O$ (c)	15748	0.79	0.30	0.80	0.79	0.36
Cu(Gly·Phe)(phen)·$3H_2O$ (d)	15723	0.76	0.34	0.82	0.99	0.21
Cu(Gly·Tyr)(bipy)·$4H_2O$ (e)	15674	0.79	0.30	0.79	0.86	0.35
Cu(Gly·Tyr)(phen)·$3H_2O$ (f)	15674	0.76	0.33	0.83	0.86	0.29
Cu(Gly·Tyr)(dmph)·$4H_2O$ (g)	15873	0.74	0.35	0.84	0.88	0.30
Cu(Gly·Gly)(bipy)·$3H_2O$ (h)	15527	0.76	0.33	0.84	0.81	0.40
Cu(Gly·Gly)(phen)·$3H_2O$ (i)	15625	0.78	0.31	0.83	0.77	0.40
Cu(Gly·Gly)(dmph)·$4H_2O$ (j)	15723	0.74	0.35	0.84	0.99	0.23

Furthermore, the axial ligand has little effect on α^2 values. The β_1^2 values for α-diimine adducts are higher than the Cu(II) dipeptides because this parameter is related to the overlap of in-plane orbitals of π-type [25]. A lowering of symmetry of the α-diimine adducts upon coordination of α-diimine ligand is in agreement with the reduction of in-plane π-overlap [36, 38, 40]. The effective distortion in these ternary complexes from regular square pyramidal geometry can be obtained by plottingϵ'^2 against α^2 [40]. This plot is given in Fig. 3. It can be inferred from this plot that the 2,2'-bipyridine adducts of Cu(II) dipeptides, 1,10-phenanthroline adducts of Cu(II) glycyl–L-alanine, Cu(II) glycyl–L-tyrosine, and Cu(II) glycylglycine, and 2,9-dimethyl-1,10-phenanthroline adducts of Cu(II) glycylglycine, which have their points above the theoretical plot, have rhombic distortion while the 1,10-phenanthroline adduct of Cu(II) glycyl–L-phenylalanine and 2,9-dimethyl-1,10-phenanthroline adduct of Cu(II) glycyl–L-tyrosine, which have points below the theoretical plot have tetrahedral distortion. The latter may be due to the

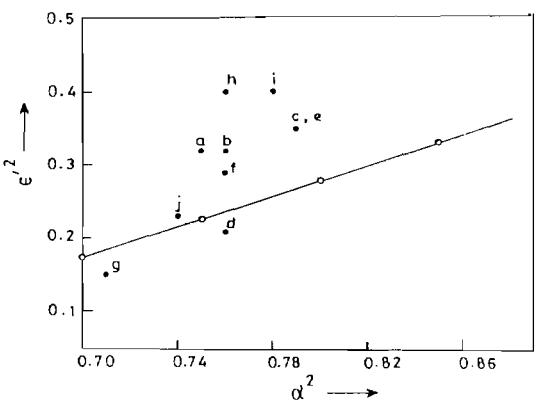


Fig. 3. Plot of α^2 against ϵ'^2 for copper(II) dipeptide complexes with α-diimine.

sterically strained structure resulting from bulkier ligands. Thus all the complexes except Cu(Gly·Phe)(phen)·$3H_2O$ and Cu(Gly·Tyr)-(dmph)·$4H_2O$ have the rhombic distorted square pyramidal geometry, while Cu(Gly·Phe)(phen)·$3H_2O$ and Cu(Gly·Tyr)(dmph)·$4H_2O$ have the tetrahedrally distorted square pyramidal geometry.

Alkaline dimethyl sulphoxide as a superoxide anion-generating system [20] in association with nitro blue tetrazolium chloride as superoxide anion scavenger [26, 41] has been used as an assay method for determining superoxide dismutase activity. Alkaline DMSO as a O_2^- generating system has the advantage that there is a marked decrease in the spontaneous dismutation rate of O_2^- due to the use of a relatively high pH combined with the low temperature and that a large amount of O_2^- is generated in solution as compared to SOD concentration. Thus, the data for SOD activity of some Cu(II) dipeptides and their adducts using the above DMSO–NBT assay method are given in Table 1. One representative example of a plot of inhibition with increase in concentration of Cu-(Gly·Gly)(bipy)·$3H_2O$ (0–200 μM) is shown in Fig. 4. The data suggest that the square pyramidal Cu(Gly·Gly)·$3H_2O$ show moderate activity. The substitution of both equatorial and axial water molecules by 2,2'-bipyridine or 1,10-phenanthroline causes enhancement of SOD activity. The same behaviour is observed if both equatorial and axial water molecules of Cu(Gly·Ala)·$2H_2O$ are substituted by 2,2'-bipyridine or 1,10-phenanthroline. Cu-(Gly·Tyr)(phen)·$3H_2O$ shows the highest SOD activity among the Cu(II) complexes studied and it is about 18 times less active than the bovine erythrocyte SOD enzyme on molar basis.

The substitution of the equatorial and axial water molecules of Cu(II) dipeptides by α-diimine ligands

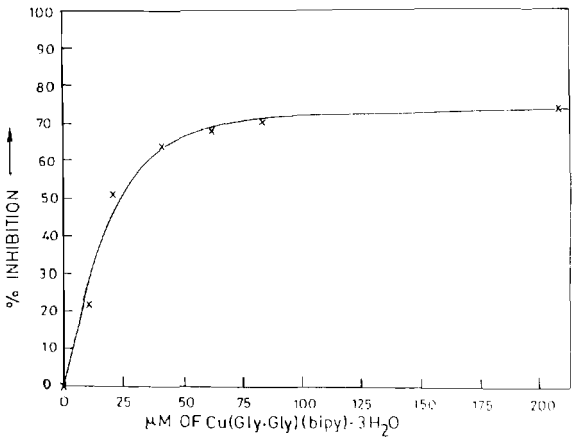


Fig. 4. A plot showing percentage inhibition of NBT reduction with increase in concentration of Cu(Gly·Gly)(bipy)·$3H_2O$ (0 to 200 μM).

gives more activity than the parent Cu(II) dipeptides. A greater interaction between superoxide ion and Cu(II) in ternary Cu(II) complexes is induced due to the stronger axial bond [42], which results in an increased catalytic activity. In addition α-diimine ligands stabilize the Cu(I) complex formed during superoxide dismutation reaction which further reacts with superoxide ion to give hydrogen peroxide. The distorted geometry of these complexes may favour the geometrical change, which is essential for the catalysis as the geometry of copper in the SOD enzyme also changes from distorted square pyramidal (for Cu(II)) to distorted tetrahedral (for Cu(I)) during catalysis [43, 44].

A comparative look at Cu(II) dipeptides and their α-diimine adducts shows that the methyl side chain of L-alanine has a deactivating effect. This may be explained by the relative inductive effect of the side chain substituents and also from the ESR parameter $g_{\parallel}$ which has the lowest value indicative of strong equatorial ligand field. The higher activity of α-diimine adducts of Cu(II) glycyl-L-tyrosine and Cu(II) glycyl-L-phenylalanine may be explained on the basis of the favourable response of π-electrons of the aromatic side chain in stabilizing the Cu(II)–O_2^- interaction. This type of interaction was also observed by Kimura and his coworkers [45] in macrocyclic polyamine Cu(II) complexes containing a pyridine ring. In addition, the hydroxyl group of tyrosine in the α-diimine adducts of Cu(II) glycyl-L-tyrosine may perhaps further stabilize the Cu(II)–O_2^- complex by hydrogen bonding. This would be similar to the role of the arginine-141 residue involved in the superoxide dismutation mechanism by the native SOD enzyme [44].

Conclusions

A series of stable and water soluble ternary complexes of Cu(II) dipeptides with α-diimine has been prepared that have a distorted square pyramidal geometry. The distorted structure of the above complexes is responsible for their good superoxide dismutase activity. The present study demonstrates that it is possible to enhance the SOD activity of Cu(II) complexes further if appropriate modifications in their coordination geometry are made.

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