C H A P T E R - II A

INTRAMOLECULAR INTERLIGAND INTERACTIONS IN MIXED - LIGAND COMPLEXES INVOLVING DIPEPTIDES AND AMINO ACIDS

INTRODUCTION

The amino acids and dipeptides are structural constituents of proteins and their complexes with the metal ions, present in the body fluid, are of biochemical importance. Although it is difficult to reproduce in vitro exact structural conditions, as existing in biosystems, it may be possible to mimic them by chosing simple models. Complexes of amino acids and oligopeptides are involved in the exchange and transport mechanism of trace metal ions in the human body [1]. Oligopeptides can be used as model compounds for such studies, because they are able to mimic to a great extent the metal binding sites of much more complicated protein molecules. For a less specific but rather general study of the metal binding of peptides, even studies of dipeptides can supply much information. As stated in chapter I, in biological systems there is strong possibility of formation of ternary complexes. Hence it is worth while to study various ternary systems involving dipeptides and amino acids.

Martin et al [2] detected the formation of two mixed species CuAB and $CuA_{-H}B$ in the system Cu-diglycylglycinate (A) - glycinate (B). Nagypal and Gergely [3,4] also studied pH metrically some binary and mixed-ligand complexes of Cu(II) with dipeptides and amino acids. The dipeptides used were glycylglycine, glycyl- DL-α-alanine, DL-α - alanyl-DL-α- alanine and the amino acids were glycine, α-alanine, β-alanine, α-amino butyric acid, norvaline, serine, threonine, ornithine, lysine, asparagine, aspartic acid, glutamine and glutamic acid. Gergely et al [5] also studied the binary complexes of copper(II) with glycyl serine, glycyl-asparagine, glycyl-aspar-

tic acid and glycyl-glutamine. The interaction of Cu^{+2} and Ni^{+2} with tryptophan (Trp) and glycyltryptophan (GlyTrp) was studied by monitoring the quenching of fluorescence due to complex formation [6]. Shelke et al [7] studied the stability of binary copper(II) complex of the dipeptide alanylphenylalanine and its ternary complexes with amino acids. Gergely et al [8] did spectrophotometric and calorimetric measurements for Cu(II), Ni(II) and Zn(II) binary and mixed ligand complexes containing serylglycine, which contains alcoholic hydroxyl group in its N-terminal side chain and L-α-alanine, β-alanine, L-aspartic acid or 2,2'bipyridyl as secondary ligands.

The formation and stability of the mixed-ligand complexes of Cu(II) with diethylenetriamine (dien) and amino acids or peptides were studied by potentiometric and conductometric techniques [9]. Binary and ternary complexes of Cu(II) with glycyl-L-tyrosine and an amino acid ester were investigated by potentiometric and spectro-photometric techniques [10].

Bhattacharya and co-workers [11] studied the interligand interactions in the ternary complexes involving mixed amino acids. The complexes studied in aqueous medium were of the type CuLL' where L=L'=tryptophan (trp), tyrosine (tyr) or phenylalanine (phe). The study was further extended to the case of ternary complexes involving 3,4-dihydroxyphenylalanine (L-dopa) as one of the ligands. It was observed that $\triangle$ log K for the above complexes were less negative or more positive. This was attributed to the possible hydrophobic interaction or hydrogen bonding between the non-coordinating aromatic side groups of the amino acids.

The dipeptide may have non-coordinated COO^- side group in the ternary complex and may undergo intramolecular interligand interactions with the

non-coordinated side groups of other ligands, like amino acids. In order to investigate the possibility of such types of interligand interactions in the ternary complexes involving dipeptides and amino acids, complexes of the type CuAL where A=glycylglycine (gg), glycyl-L-alanine (ga) or glycyl-L-leucine (gl) and L= α-alanine (ala), phenylalanine (phe), tryptophan (trp), tyrosine (tyr) and 3,4 dihydroxy phenylalanine (L-dopa) have been studied in the present chapter.

EXPERIMENTAL

Chemicals : The ligands glycylglycine, glycyl-L-alanine, glycyl-L-leucine (Sigma), α-alanine, phenylalanine (BDH), tryptophan (Fluka), tyrosine (E. Merck) and 3, 4 - dihydroxyphenylalanine (Sigma) were of A.R. grade. All these reagents were used as received. 1,4 -Dioxane was purified according to literature method [12]. In all the experiments conductivity water was used.

Copper perchlorate was prepared from analytically pure copper carbonate by treatment with 70% perchloric acid (A.R). The resulting solid was vacuum filtered, washed with ethanol, till it was free from excess acid and recrystallized several times from ethanol.

Stock solution of copper perchlorate, perchloric acid, sodium hydroxide and sodium perchlorate were all prepared in (carbonate free double distilled) deionized water. Copper perchlorate solution was standardized by iodometric method, as well as by complexometric titration with standard EDTA solution, using pyrocatechol violet as indicator [13]. Carbonate free sodium hydroxide solution was prepared according to the literature method [14] and standardized by potentiometric titration with standard oxalic acid solution. Standard perchloric acid solution was prepared from A.R. 70% acid by proper dilution and titration with standard alkali.

Fresh solution of ligands having appropriate concentration were prepared every time from the pure compounds in deionized water.

Potentiometric determination of the stability constants :

The solutions containing appropriate amounts of standard perchloric acid, sodium perchlorate, ligand and metal solutions were titrated, potentiometrically, against standard 0.20 M sodium hydroxide solution. In all the cases acid concentration was kept 2.00×10^{-2} M and the total ionic strength (I) of the solutions was maintained at 0.2 M with standard sodium perchlorate solution. All the titrations were carried out in 50% (1:1, v/v) water-dioxane medium. Temperature was maintained at 30°C during the progress of the titrations.

The titrations were carried out using a digital pH meter (DIGICHEM-8201) having an accuracy of ± 0.01 and a Toshniwal combination glass electrode.

Observed values of the pH were corrected to get the concentration of hydrogen ion in the solution. For this, pH values of standard perchloric acid solutions have been determined under the experimental conditions and compared with theoretical values. The difference takes into account liquid junction potential across the membrane and effect of the ionic strength of the medium [15]. The constant difference has been considered as correction for other titration data.

The titration data has been processed to get the refined values of the formation constants using the computer program SCOGS (Stability Constants of Generalized Species) [16-18], which employs the conventional non-linear least square approach. For a mixture of maximum two metals A, B and two ligands S, T, association constants may in principle be calcula-

ted with the program for any species j which can be described by the general formula.

$$A_{A_j}\ B_{B_j}\ S_{S_j}\ T_{T_j}\ (OH)_{W_j}$$

Where A_j, B_j, S_j, T_j are positive integers or zero, and W_j is a positive integer (for a hydrolysed species), zero, or a negative integer (for a protonated species). The calculated practical overall formation constant β_j can be given by the expression

$$\beta_j = \frac{[A_{A_j}\ B_{B_j}\ S_{S_j}\ T_{T_j}\ (OH)_{W_j}]}{[A]^{A_j}\ [B]^{B_j}\ [S]^{S_j}\ [T]^{T_j}\ \{OH\}^{W_j}}$$

where square brackets [ ] denote concentrations and braces { } denote activities.

While treating the titration data with SCOGS, activity coefficient has been considered to be equal to 1 [19] and the value of the ionic product of water in 50% (v/v) water - dioxane was considered to be 1.419×10^{-16} [19,20].

The proton - ligand formation constant of the dipeptides AH_2 and AH and the formation constant of the binary complexes CuA and CuA_{-H} were determined in 50% (v/v) water - dioxane medium. For the amino acids also the proton - ligand formation constants LH_2 and LH and the binary constants CuL and CuL_2 were refined. The values have been tabulated in Tables 2A.1 and 2A.2, respectively. These refined values were used as

fixed parameters for the refinement of the formation constants of the mixed ligand complexes CuAL and $CuA_{-H}L$.

In order to determine the formation constant of the ternary complexes, the following sets of solutions (50 cc) having Cu, A and L in 1:1:1 and 1:1:2 ratios were prepared and titrated against standard sodium hydroxide solution:

(i) 0.02 M $HClO_4$, 0.004 M ligand A, 0.004 M ligand L, 0.004 M metal perchlorate and 0.168 M sodium perchlorate.

(ii) 0.02 M $HClO_4$, 0.004 M ligand A, 0.008 M ligand L, 0.004 M metal perchlorate and 0.164 M sodium perchlorate.

The above sets were kept for nearly 20 minutes at room temp. to attain equilibrium and then all the titrations were carried out in nitrogen atmosphere. Titrations of each set were carried out twice to check the reproducibility of the data. The evaluation of the formation constants of the ternary copper(II) - dipetide - amino acid complexes was done by considering simultaneous existence of all the species AH_2, AH, A, LH_2, LH, L, Cu, CuA, CuA_{-H}, CuL, CuL_2, CuAL and $CuA_{-H}L$. The values were refined by the computer program SCOGS and are tabulated in Table 2A.3. The pH titration curves have been presented in figs 2A.1 to 2A.5. The distribution of various binary and ternary species as a function of pH has been shown in fig 2A.6 for the complex Copper(II) - glycylglycine-tyrosine.

Spectral Studies : Electronic spectra were recorded in 50% dioxane-water medium (1:1, v/v) on shimadzu UV 240. The spectral data are presented in Table 2A.4. The spectra of mixed-ligand complexes were recorded by mixing Cu:A:L in 1:1:1 ratio. Solution of 10^{-2} mol/lit were used for visible region. The spectra were recorded at different pH as shown in fig. 2A.7.

Table 2A.1 : Proton-Ligand formation constants of the Dipeptides and their corresponding Binary constants in 50% Dioxan-Water (1:1, v/v) medium at 0.2 M $NaClO_4$ and 30°C. Standard deviation (σ-β) are given in parentheses.

Ligands	pK_1^H	pK_2^H	$\log K_{CuA}^{Cu}$	$\log K_{CuA}^{CuA_{-H}}$
Glycylglycine (gg)	7.80	4.05	6.23	3.92
	(0.01)	(0.01)	(0.05)	(0.01)
Glycyl-L-alanine (ga)	7.91	4.56	6.47	3.92
	(0.02)	(0.03)	(0.07)	(0.02)
Glycyl-L-leucine (gl)	7.97	4.64	6.82	4.77
	(0.03)	(0.04)	(0.05)	(0.04)

Table 2A.2 : Proton-Ligand formation constants and Binary complex formation constants of the Amino Acids in 50% Dioxane-Water medium (1:1, v/v) at 0.2 M $NaClO_4$ and 30°C. Standard deviation ($\sigma\beta$) is given in parentheses.

Ligands	pK_1^H	pK_2^H	$logK_{CuL}^{Cu}$	$LogK'_{CuL_2}^{CuL}$
α-Alanine (ala)	9.56	3.18	9.16	7.00
	(0.01)	(0.01)	(0.07)	(0.10)
Phenylalanine (phe)	8.98	3.11	8.96	7.37
	(0.00)	(0.01)	(0.04)	(0.05)
Tryptophan (trp)	9.24	3.20	9.12	7.82
	(0.00)	(0.01)	(0.04)	(0.04)
Tyrosine (tyr)	9.06	3.14	8.92	7.35
	(0.00)	(0.01)	(0.06)	(0.07)
3,4-dihydroxy-phenylalanine (L-dopa)	8.93	3.13	8.99	7.35
	(0.01)	(0.01)	(0.01)	(0.01)

Table 2A.3 : Formation constants of mixed-ligand complexes and $\triangle$ logK in 50% water-dioxane (1:1, v/v) medium, I = 0.2 M ($NaClO_4$) at 30°C. Standard deviation ($\sigma \sim \beta$) are given in parentheses.

Complex	$LogK^{Cu}_{CuAL}$	$\triangle$LogK	$LogK^{CuA_{-H}L}_{CuAL}$
Cu-gg-ala	14.59	- 0.90	7.88
	(0.05)		(0.04)
Cu-gg-phe	14.58	- 0.71	8.31
	(0.04)		(0.06)
Cu-gg-trp	15.04	- 0.42	7.94
	(0.02)		(0.10)
Cu-gg-tyr	14.89	- 0.36	7.91
	(0.05)		(0.07)
Cu-gg-dopa	14.95	- 0.37	6.89
	(0.10)		(0.10)
Cu-ga-ala	15.24	- 0.39	8.53
	(0.09)		(0.11)
Cu-ga-phe	15.48	+ 0.05	8.19
	(0.06)		(0.10)
Cu-ga-trp	15.81	+ 0.21	8.19
	(0.06)		(0.18)

Table 2A.3 (Contd.)

Cu-ga-tyr	15.60	+ 0.21	8.27
	(0.07)		(0.10)
Cu-ga-dopa	15.61	+ 0.16	7.05
	(0.05)		(0.06)
Cu-gl-ala	15.19	- 0.80	8.68
	(0.09)		(0.11)
Cu-gl-phe	14.95	- 0.83	8.28
	(0.04)		(0.08)
Cu-gl-trp	15.34	- 0.61	8.32
	(0.06)		(0.20)
Cu-gl-tyr	15.09	- 0.65	8.40
	(0.07)		(0.09)
Cu-gl-dopa	15.18	- 0.63	6.81
	(0.10)		(0.11)

Table 2A.4 : Ligand field bands of (CuAL) and ($CuA_{-H}L$) complexes in 50% water-dioxane (1:1, v/v) medium.

Complex	pH	Observed value (nm)
Cu-gg-ala	4.50	642.6
Cu-gg-tyr	4.50	634.4
Cu-gg-ala	6.05	625.0
Cu-gg-tyr	6.05	614.8
Cu-gg-ala	7.80	616.8
Cu-gg-tyr	7.80	612.6

Fig. 2A.1 Potentiometric titration curves of 50% (v/v) water - dioxan solutions containing metal ions gg, ga or gl and ala (each 4.0×10^{-3} M).

(1) Cu^{2+} + gg + ala

(2) Cu^{2+} + ga + ala

(3) Cu^{2+} + gl + ala

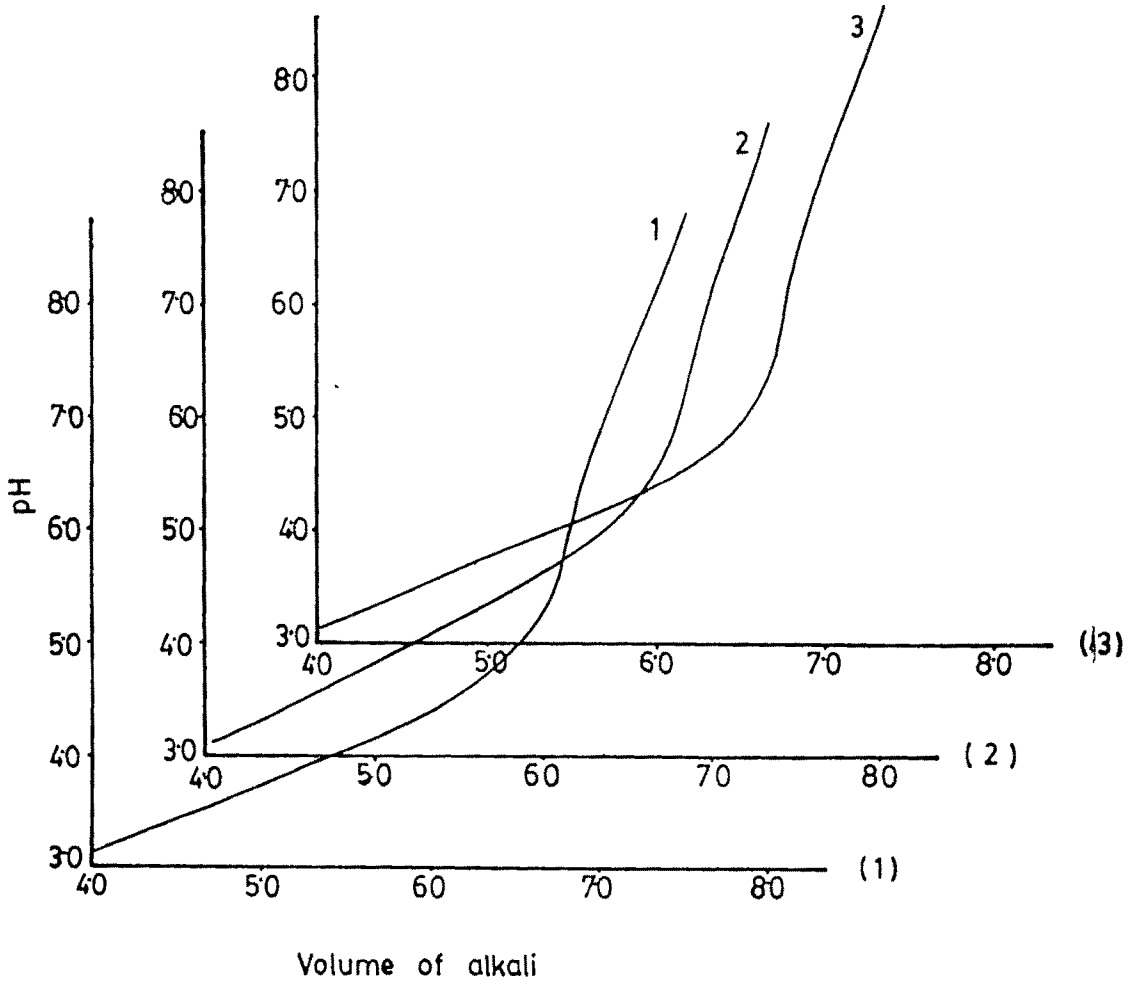


Fig. 2A.2 Potentiometric titration curves of 50% (v/v) water - dioxan solution containing metal ions gg, ga or gl and phe (each 4.0×10^{-3} M).
(1) Cu^{2+} + gg + phe
(2) Cu^{2+} + ga + phe
(3) Cu^{2+} + gl + phe

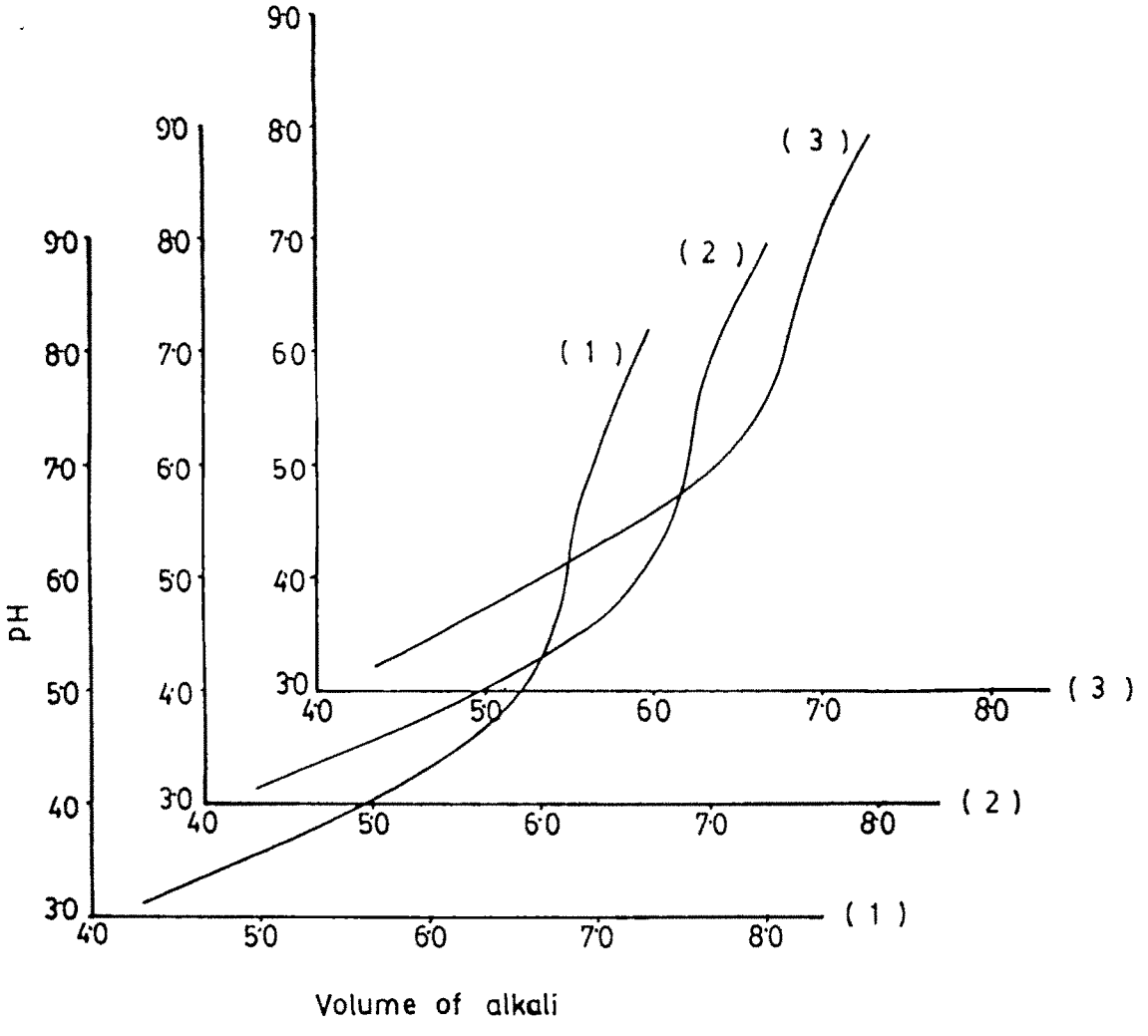


Fig. 2A.3 Potentiometric titration curves of 50% (v/v) water - dioxan solutions containing metal ions gg, ga or gl and trp (each 4.0×10^{-3} M).
(1) Cu^{2+} + gg + trp
(2) Cu^{2+} + ga + trp
(3) Cu^{2+} + gl + trp

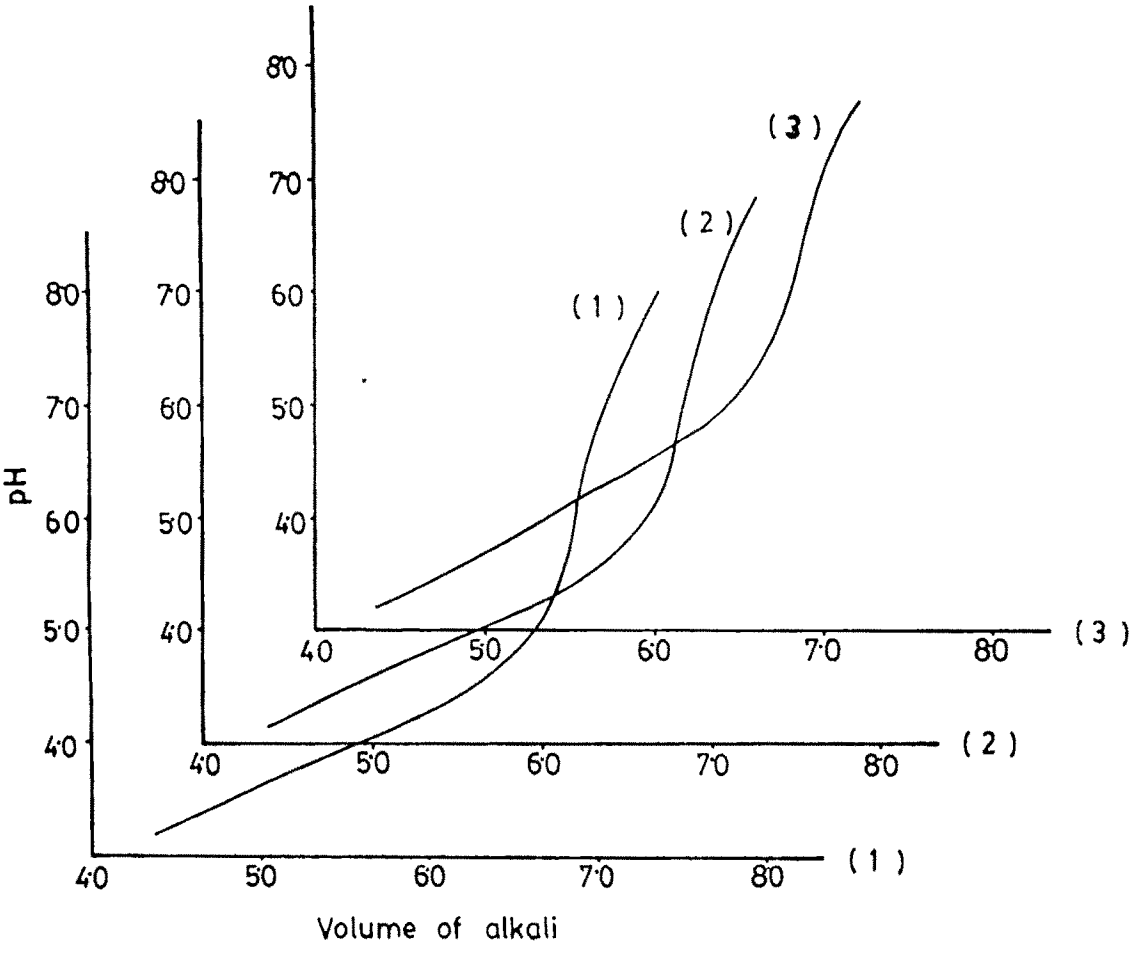


Fig. 2A.4 Potentiometric titration curves of 50% (v/v) water –dioxan solutions containing metal ions gg, ga or gl and tyr (each 4.0×10^{-3} M).

(1) Cu^{2+} + gg + tyr

(2) Cu^{2+} + ga + tyr

(3) Cu^{2+} + gl + tyr

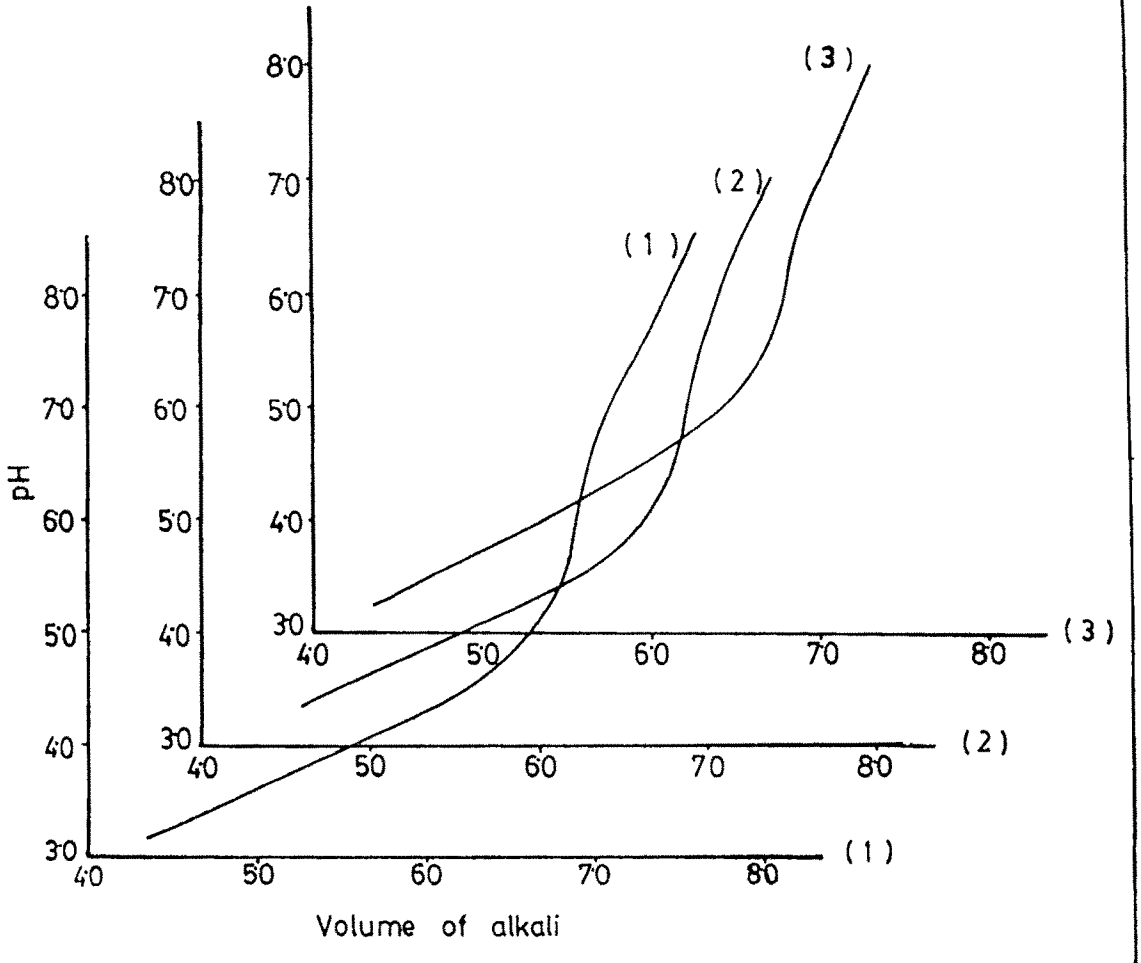


Fig. 2A.5 Potentiometric titration curves of 50% (v/v) water - dioxan solutions containing metal ions gg, ga or gl and L - dopa (each 4.0×10^{-3} M).
(1) Cu^{2+} + gg + L - dopa
(2) Cu^{2+} + ga + L - dopa
(3) Cu^{2+} + gl + L - dopa

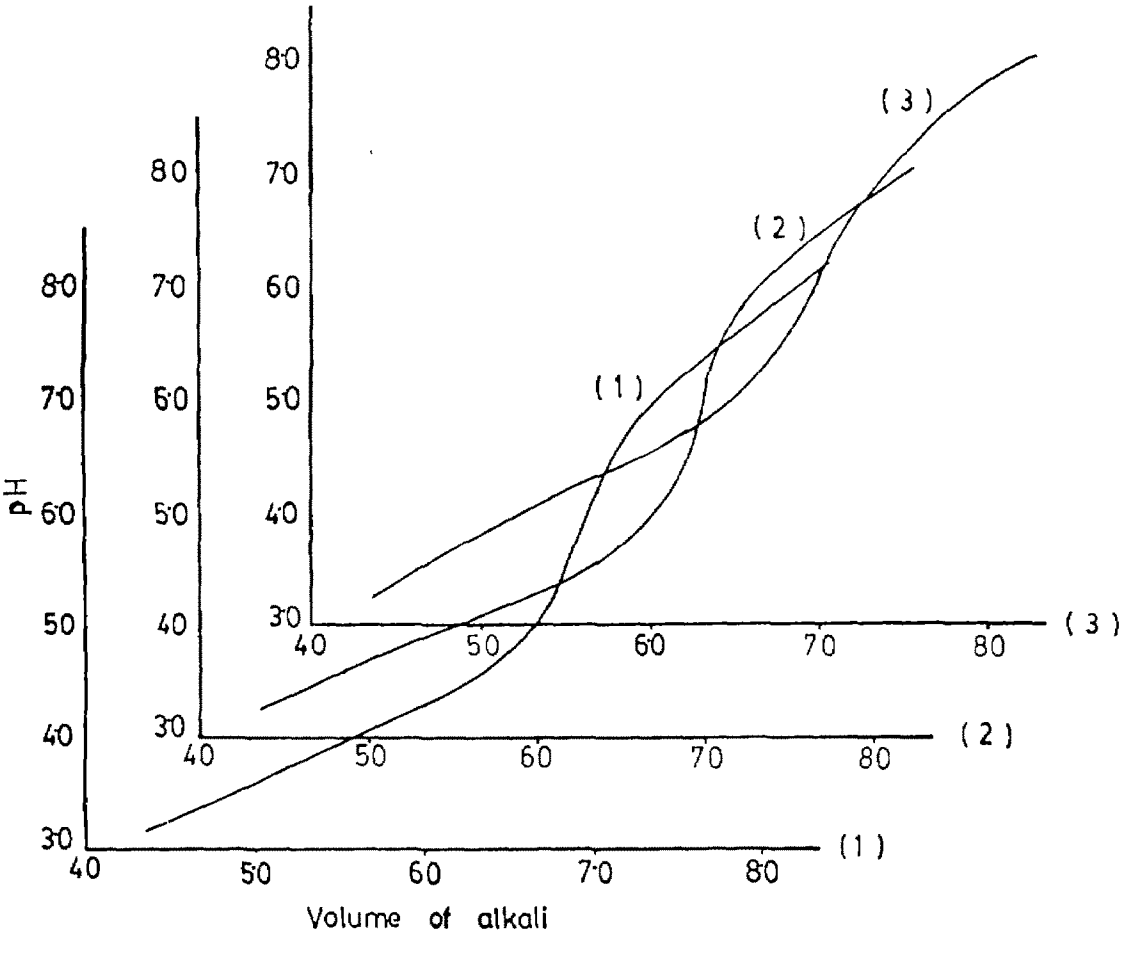


Fig. 2A.6 Species Distribution for the Copper (II) - Glycylglycine (A) - tyrosine (L) ternary system at Metal : A : L ratio of 1:1:1 ; (1) Unbound copper (II), (2) (CuA), (3) (CuA_{-H}), (4) (CuL), (5) (CuAL) and (6) $CuA_{-H}L$.

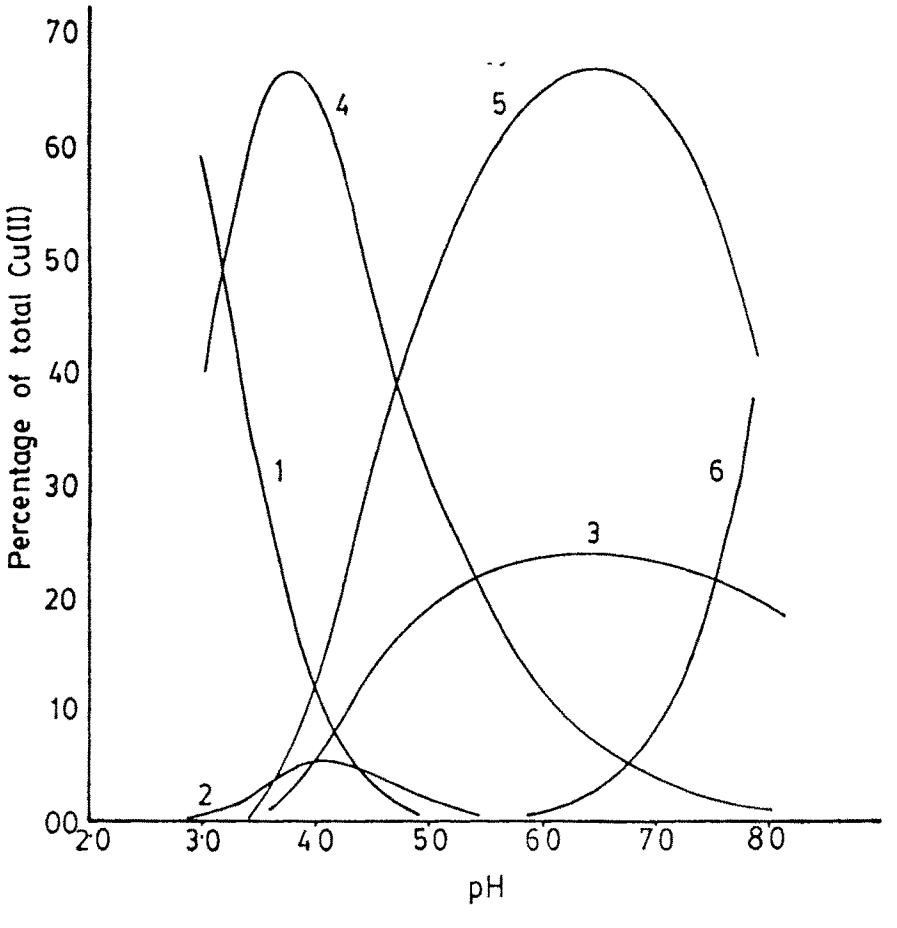
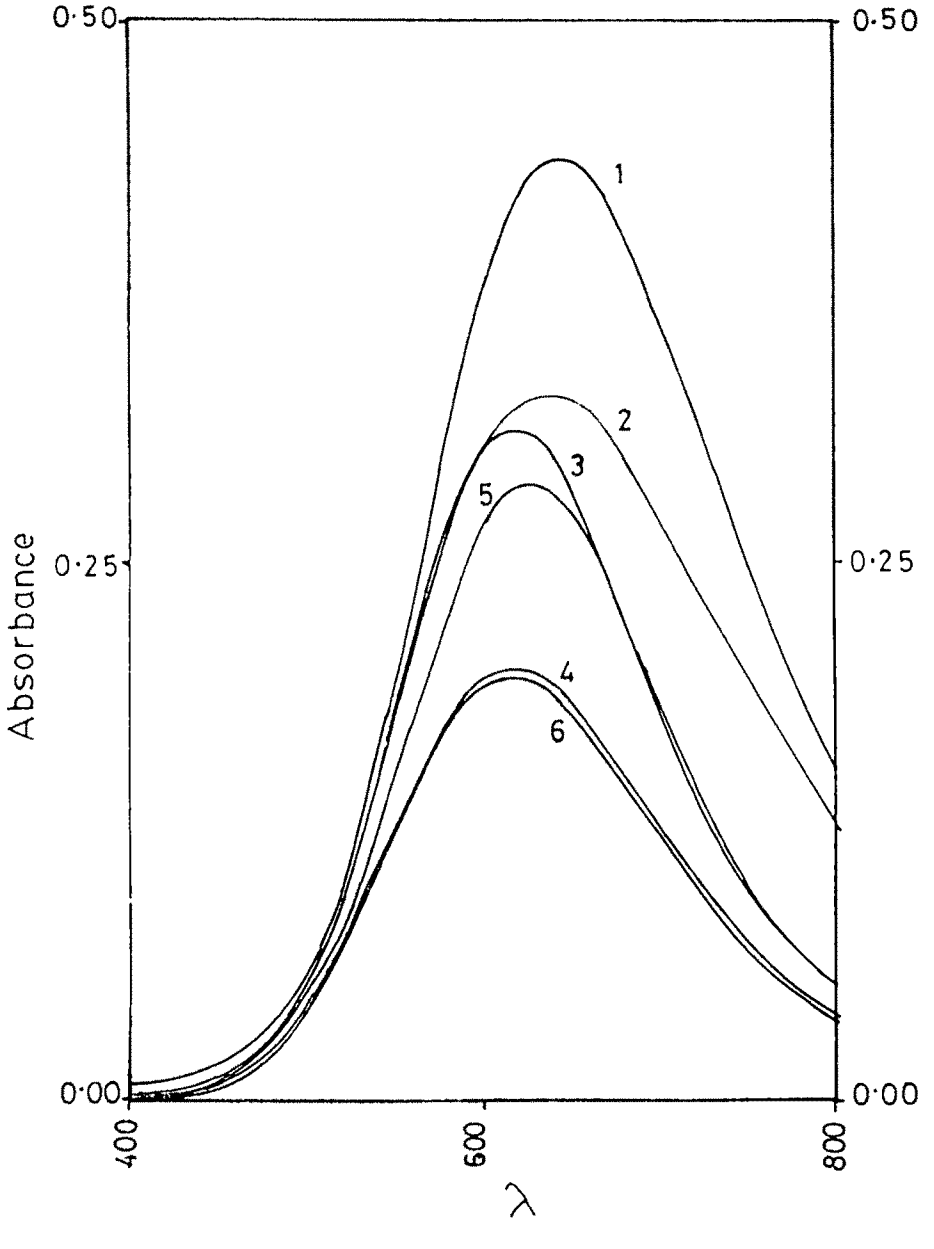


Fig. 2A.7 Electronic spectra of (1) Cu-gg-ala ; pH = 4.5 (2) Cu-gg-tyr ; pH = 4.5, (3) Cu-gg-ala ; pH = 6.05 (4) Cu-gg-tyr ; pH = 6.05, (5) Cu-gg-ala ; pH = 7.80 and (6) Cu-gg-tyr ; pH = 7.80, in 50% (1:1, v/v) water - dioxan medium.



RESULTS AND DISCUSSIONS

The equilibria corresponding to the binary constants CuA and CuA_{-H} of the dipeptides are as follows :

$$Cu + A \rightleftharpoons CuA \qquad (2A.1)$$

$$K^{Cu}_{CuA} = \frac{[\ CuA\ ]}{[Cu][A]} \qquad (2A.2)$$

$$CuA_{-H} + H \rightleftharpoons CuA \qquad (2A.3)$$

$$K^{CuA_{-H}}_{CuA} = \frac{[\ CuA\ ]}{[CuA_{-H}]\ [H]} \qquad (2A.4)$$

The second equalibrium constant K^{CuA-H}_{CuA} is the protonation constant of the species CuA_{-H}. As the peptide -NH does not undergo deprotonation in the free ligand, it is not possible to find the protonation constant of the peptide N-H. Hence it is not possible to calculate the formation constant of the complex CuA_{-H}. The peptide -NH undergoes deprotonation only after coordination with Cu^{+2} during the formation of CuA_{-H}. Therefore, only the protonation constant of the species CuA_{-H}, as shown in equation (2A.4) above can be calculated.

Species distribution curves (fig 2A.6) show the simultaneous formation of CuA and CuAL species. The ternary complex CuAL is formed in the pH range 3.50-6.00. At pH $\sim$ 6.00 the concentration of the above species attains a maximum value of $\sim$ 75%. The formation of the deprotonated ternary species $CuA_{-H}L$ starts from $\sim$ 6.00 pH and its concentration rises steeply with the rise in pH.

Formation and Stability of CuAL Complexes :

The constant for the ternary species CuAL correspond to the following equilibria :

$$Cu + A + L \rightleftharpoons CuAL \qquad (2A.5)$$

$$K^{Cu}_{CuAL} = \frac{[\ CuAL\ ]}{[Cu][A][L]} \qquad (2A.6)$$

In the CuAL complex the dipeptide A is coordinated from the amino group and peptide C=O, its carboxylate part remaining uncoordinated. The dipeptide contains a single negative charge. The amino acid L is coordinated through its amino nitrogen and carboxylate oxygen and also contains a single negative charge. In the case of the CuAL complexes where L = α -alanine or phenylalanine, $\triangle$ log K was found to be negative. This can be accounted for by considering the electrostatic repulsion between the amino acid anion and the dipeptide anion (fig. 2A.8). However, for L=tryptophan or tyrosine, it was observed that $\triangle$ log K was less negative or more positive compared to alanine or phenylalanine. The amino acids, tryptophan or tyrosine are coordinated from their amino carboxylate end, as in case of α -alanine. However, the indole and hydroxy phenyl group of tryptophan and tyrosine, respectively, are free. The less negative or more positive $\triangle$log K values for these complexes may be accounted for by considering the possibility of formation of hydrogen bond between the indole -NH of tryptophan or the hydroxyphenyl -OH group of tyrosine and the free carboxylate oxygen of the dipeptides (fig 2A.9). Since there is no such groups present in α-alanine or phenylalanine, hydrogen bonding is not possible in these complexes. Hence more negative or less positive value of $\triangle$ log K is observed.

L-dopa is ambidentate in nature [21-23]. For copper(II) complexes it has been observed that at a low pH (?-5) dopa coordinates via the amino carboxylate end, and above pH 6 both amino carboxylate and pyrocatecholate are involved in binding with copper(II), resulting in polymeric species. However, it was observed that in the copper(II) - dipeptide - L - dopa system, L - dopa coordinates from its amino carboxylate end over the entire pH range 4.0 - 8.0. It was also found that L dopa forms a stable complex with copper(II) - dipeptide and the $\triangle$ log K values for these complexes were found to be less negative or more positive compared to the complexes involving α-alanine or phenylalanine. The higher stability of the ternary complexes involving L-dopa is attributed to the possible formation of hydrogen bond between the -OH of hydroxyphenyl group of L-dopa and the free carboxylate oxygen of the dipeptide as in the case of copper(II) - dipeptide - tyrosine complexes (Fig.2A.10).

Since in the complexes involving copper(II) - dipeptides - L dopa, dopa coordinates from the amino carboxylate end in the whole pH range 4-8, it can be suggested that dopa administered in the form of these complexes will not undergo decarboxylation easily and will help in providing a greater supply of L-dopa to the brain, as observed for metal chelates of dopa coordinated at the amino carboxylate end [21].

It is interesting to compare the $\triangle$ log K values of the CuAL species when A = gg, ga or gl. It is found that $\triangle$ log K is more negative for the (CuglL) species than for (CuggL) species. This may be because of the bulky $CH_2CH(CH_3)_2$ group in the neighbourhood of the free carboxylate in the dipeptide, which hinders its participation in the formation of hydrogen bonds with the un-coordinated indole or hydroxyphenyl moiety of the

amino acids. Again, comparison of $\triangle$ log K values for the (CuggL) and (CugaL) species reveals that (CugaL) species are comparatively more stable than (CuggL) species. This is probably because the $-CH_3$ group adjacent to the carboxylate group in ga increases the electron density on the carboxylate oxygen, thereby making possible the formation of a strong hydrogen bond with the amino acid with side group.

Formation and Stability of $CuA_{-H}L$ Complexes :

For the ternary species $CuA_{-H}L$, only the protonation constant can be determined. The protonation constant is represented by the following equations :

$$CuA_{-H}L + H \rightleftharpoons CuAL \qquad (2A.7)$$

$$K^{CuA_{-H}L}_{CuAL} = \frac{[\ CuAL\ ]}{[CuA_{-H}L]\ [H]} \qquad (2A.8)$$

Hence, the deprotonation of the dipeptide A in the ternary complex CuAL can be compared with the deprotonation in the binary complex CuA. It is found that deprotonation of the peptide N-H in CuAL takes place at a higher pH compared to deprotonation in the binary complex CuA, i.e., the deprotonation is reduced in the ternary complex, compared to the binary complex. This can be accounted for by considering two reasons. First, the dipeptide on coordination from peptide -NH undergoes deprotonation, resulting in the formation of a dianion with COO^- and peptide N^-. The amino acid (L) also has a negative charge on its COO^- group. During the formation of $CuA_{-H}L$, there is a strong repulsion between the amino acid anion and the dipeptide dianion which inhibits the coordination of the dipeptide, from the peptide nitrogen and hence reduces the deprotonation.

Second, in the copper(II) dipeptide binary complex at high pH, the peptide group gets deprotonated and the species CuA_{-H} is formed, where the coordination is from amino nitrogen, peptide nitrogen and carboxylate oxygen, the three atoms occupying the three equatorial positions (fig.2A.11). In the ternary complex $CuA_{-H}L$, two of the equatorial positions will be occupied by the amino carboxylate group of the amino acid (L) so that the tridentate dipeptide (A_{-H}) has to occupy one axial and two equatorial positions. Alternately the amino acid may occupy one equatorial and one axial position and the dipeptide the three equatorial positions (fig.2A.12). In either of the above cases, occupation of the axial position will destabilize the corresponding copper(II) complex because of Jahn-Teller distortion [24]. This may also inhibit the coordination of the dipeptide from the peptide nitrogen, thus resulting in the deprotonation at higher pH in the ternary $CuA_{-H}L$ complex, compared to binary CuA_{-H} complex.

Electronic spectra of ternary complexes :

Electronic spectra of copper(II) -dipeptide-amino acid complexes show the formation of two different ternary complexes at different pH. The λ max observed at a low pH ($\sim$4.5), where the coordination of the dipeptide is from amino nitrogen and peptide oxygen, shifts to a high frequency (lower wave length) region as the pH of the solution is raised, due to change in coordination of the dipeptide to amino nitrogen and peptide nitrogen, resulting in substitution of strong field (nitrogen) for weak field (oxygen) donor.

It was also observed (fig.2A.7) that the ligand field band for the ternary complex CuAL appears at a high frequency region or low wave

length region (614.8 nm) when L = tyrosine, compared to α-alanine complex (625.0 nm). This is because there is hydrogen bonding between the two ligands in the temary complex involving tyrosine. This brings the two ligands closer to each other and hence a strong ligand field is produced, resulting in the shift of the absorption band to higher frequency region.

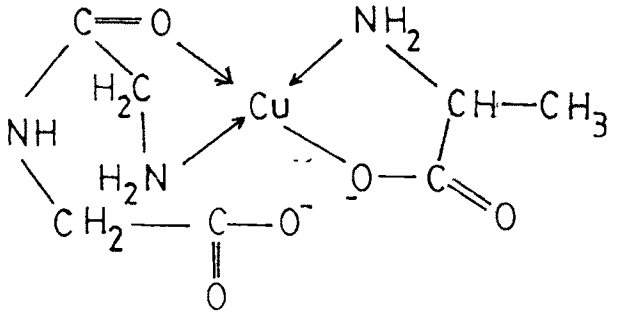


Fig. 2A·8

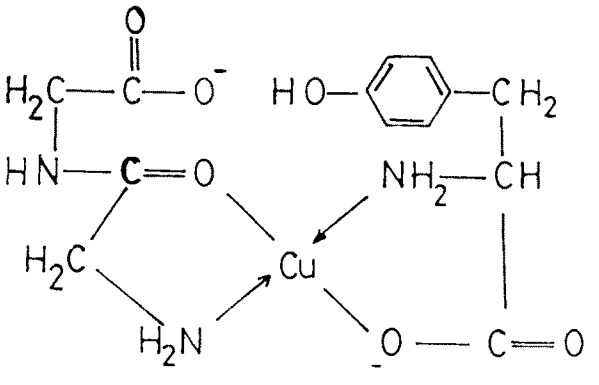
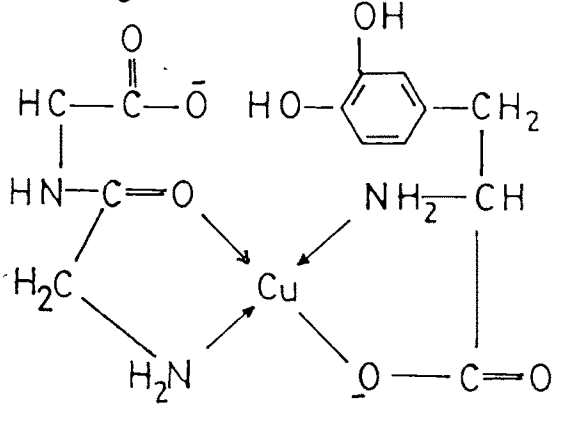


Fig. 2A·9



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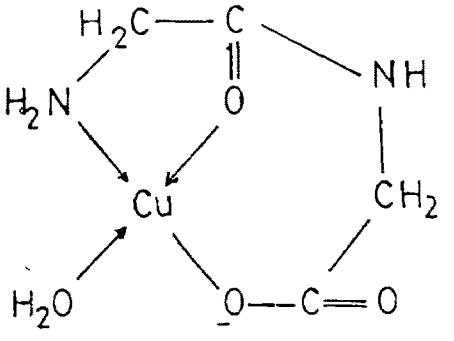

Fig 2A·11

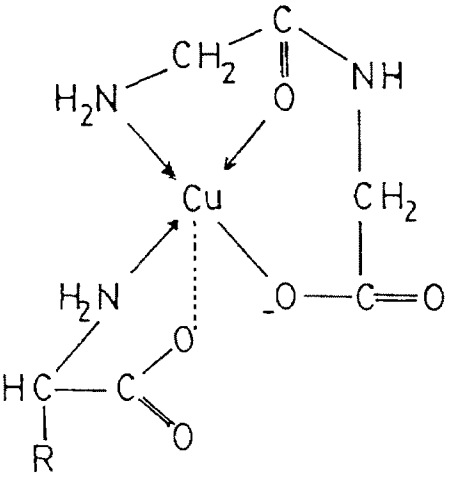

Fig 2A·12

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CHAPTER - II B

INTERLIGAND INTERACTIONS IN MIXED-LIGAND COMPLEXES INVOLVING DIPEPTIDES AND HISTIDINE

INTRODUCTION

Histidine is a tridentate ligand having an amino, imidazole and a carboxylate group as metal binding sites. With metal lons having a square planar coordination sites, histidine binding is either glycine like or histamine like [1,2]. In copper(II)-histidine binary system, there is possibility of formation of two types of 1:1 complexes, $[CuHHist]^{+2}$, where coordination is through amino nitrogen and carboxylate oxygen, the imidazole nitrogen remaining uncoordinated and protonated and $[CuHist]^{+}$, where histidine coordinates through imidazole nitrogen and amino nitrogen, and there is weak coordination from the carboxylate group [3,4]. A third type of coordination was suggested earlier [2,5] considering that in the species [CuHHist], coordination is from imidazole nitrogen and carboxylate oxygen, the amino nitrogen remaining uncoordinated and protonated. However, this will give rise to a less stable seven-membered ring, and hence its formation is unlikely.

Pettit and co-workers [6] determined the formation constants of various possible binary copper-histidine species in solution. They observed that in the pH range 1.8 - 3.2, there is formation of [CuHHist] species involving glycine like coordination, whereas imidazole nitrogen remains protonated. At a pH range 3.5 - 4.7, the species [CuHist] is formed with coordination from amino nitrogen and imidazole nitrogen, with possibility of a weak coordination of carboxylate oxygen also. At a still higher pH range (4.1 - 6.0) the mixed species [Cu(HHist)(Hist)] is formed, where one histidine molecule binds in glycine like manner and another histamine like. The above modes of coordination of histidine were also confirmed by spectrophotometric studies [7]. Formation of the species $[CuHist_{-H}]$

has also been shown to occur at very high pH (>9.0) due to the deprotonation of the imidazole -NH [8].

The enzymes containing the histidyl residues are mostly involved in oxygen transport [9-12], electron transfer [13-14] or oxygen activation [14-18], processes. Crystal structure determination, spectroscopic and various other techniques show that the imidazole moiety binds to Cu(II) in sperm whale myoglobin [19], bovine serum albumin [20], haemocyanin [21], ceruloplasmin and ribonuclease as well as in a number of other biomolecules [22]. Hence, the interaction of derivatives of histidine with metal ions has been extensively studied particularly in Copper (II) complexes [3,23,24].

Cobalt(II), Nickel(II) and Zinc(II) complexes of glycyl-L-histidine, L-histidylglycine and L-carnosine were studied by measuring pH in 0.2 mol dm^{-3} KCl solutions at 25°C [25]. The mixed ligand complexes of glycine, glycyl-glycine and histidine were also studied with the above dipeptides as primary ligands. Copper(II) complexes of histidine containing dipeptides, glycyl-L-histidyl-glycine, glycyl-glycyl-L-histidine, L-pyroglutamyl-L-histidyl-L-prolinamide and L-pyroglutamyl-L-histidine methyl ester were studied by pH-metric and spectrophotometric method [26].

The pH dependence of the formation of complexes between copper(II) and the peptide glycyl-histidine was studied for metal-ligand ratios of 1:1 and 1:2 by ESR [27]. Proton-ligand and Ni(II),

Co(II), Cu(II) and Zn(II) complex stability constants of the histidine containing dipeptides, histidyl phenylalanine and histidyl tyrosine, were determined at ionic strength 0.2 and 298°K by pH-metric, spectrophotometric, ESR and calorimetric methods [28]. It was observed that the amide group do not deprotonate and coordinate to the metal ions. The ratio of the 1:1 and 1:2 metal:ligand stability constants for Co(II) and Ni(II) indicate metal ion aromatic side chain interaction.

Cyclic dipeptides with amino residues containing complexing side-chain groups can coordinate to metal ions in a way that mimics the coordination sites of enzymes. To obtain an improved understanding of the complexing properties of cyclopeptides involved in biological systems, the interactions in aqueous solution of protons and copper(II) with cyclo-(L-histidyl-L-histidyl) were studied by potentiometric pH titration and calorimetry[29].

Homo and heteronuclear complexes of copper(II) and cadmium (II) with L-histidyl glycine have been investigated by potentiometry, visible spectrophotometry and calorimetry in aqueous media, at T=25°C and I=0.1 mol dm^{-3}(KNO_3)[30].

Ternary complexes of copper(II) containing L-histidine as one of the ligands are of biological significance. Kruck and Sarkar [31] suggested that the ternary complex copper(II)-L-histidine-albumin acts as an intermediate in the exchange of copper(II) in blood between a macromolecule such as albumin and a low molecular weight substance like an amino acid. The exchange-

able portion of copper(II) in blood plasma was shown to be mainly in the form of ternary complex [Copper(II) - asparginato-histidinato], [Copper(II) - asparginato-threoninato] and [Copper(II)-histidinato-threoninato].

Bhattacharya and co-workers [32] studied the mixed-ligand complexes of copper(II) involving histidine (A) and another amino acid (L). The amino acids selected were tryptophan, phenylalanine, tyrosine and 3,4 - dihydroxyphenylalanine (L-dopa). Two types of ternary complexes (CuAHL) and (CuAL) were shown to be formed. The complex (CuAHL) was found to be stable due to stacking interactions between the free protonated imidazole of histidine and non-coordinated side groups of the amino acids. The complex (CuAL) was also found to be stable due to hydrogen bonding between COO^- group of A and -OH or -NH groups of tyrosine, L-dopa or tryptophan. Brookes and Pettit [33] studied the copper-histidine-L complexes where L=glycyl-L-valine or valylvaline.

In order to further investigate the possibility of intramolecular interligand interactions in ternary complexes involving histidine, in the present chapter, complexes involving the dipepdides (A), gg, ga and gl as described in chapter II A, and histidine (L) (hist) have been studied in aqueous medium at initially constant lonic strength of 0.2 M at temp. 25° C.

EXPERIMENTAL

The required solutions such as metal perchlorate, sodium hydroxide, perchloric acid and sodium perchlorate were prepared and standardized in the same way as detailed in chapter II A. The dipeptides (gg, ga and gl) were of the same quality as mentioned in chapter II A. Histidine was obtained from sigma and was of A.R.grade.

Potentiometric titrations were carried out in aqueous medium using ORION IONALYZER with an accuracy of ± 0.001. The values of proton-ligand formation constant of the dipeptides and metal ligand formation constant of the binary complexes with copper(II) were determined in aqueous medium. The values were refined using the computer program SCOGS. The species considered were AH_2,AH,A,Cu,CuA and CuA_{-H}. These values have been tabulated in table 2B.1 and are in agreement with the values reported earlier [7]. The proton-ligand formation constant of histidine and formation constant of copper(II)-histidine complexes were also refined under identical conditions. The species considered were LH_3,LH_2,LH,L,Cu,CuLH,CuL,CuLHL and CuL_2. The values are tabulated in Table 2B.2 and are in agreement with the values reported earlier. These refined values were used as fixed parameters for the refinement of the formation constants of the mixed ligand complexes.

The formation constants for the ternary systems were computed from titrations, in which total concentrations of metal, ligand A and ligand L were in 1:1:1 and 1:1:2 ratios. The evaluation of the formation constants of the mixed-ligand complexes was done considering the simultaneous formation of all the possible binary and ternary species AH_2, AH, A, LH_3,LH_2,LH, L,Cu,CuA, CuA_{-H}, CuLH, CuL, CuL_2, CuALH, CuAL and $CuA_{-H}L$. The values for the formation constants have been presented in table 2B.3. The titration

data for the ternary systems are given in fig .2B.1. The distribution of various binary and ternary species (as percentage of total metal) as a function of pH has been shown in fig.2B.2 for the complex copper(II) - glycylglycine-histidine.

Table 2B.1 : Proton-ligand formation constants of the dipeptides (A) and formation constants of their binary copper(II) complexes in aqueous medium at 0.2M $NaClO_4$ and 25°C with standard deviation ($\sigma \sim \beta$) in parentheses.

Ligands	pK_1^H	pK_2^H	$\log K_{CuA}^{Cu}$	$\log K_{CuA}^{CuA-H}$
Glycylglycine (gg)	7.99	3.04	5.78	4.47
	(0.02)	(0.03)	(0.19)	(0.06)
Glycyl-L-alanine(ga)	8.07	3.03	5.84	4.41
	(0.03)	(0.04)	(0.22)	(0.07)
Glycyl-L-leucine(gl)	8.07	3.10	5.93	4.86
	(0.03)	(0.04)	(0.12)	(0.06)

Table 2B.2 : Proton-ligand formation constant of histidine (hist)(L) and formation constant of binary copper(II) complexes in aqueous medium at 0.2M ($NaClO_4$) and 25°C with standard deviation ($\sigma \sim \beta$) in parentheses.

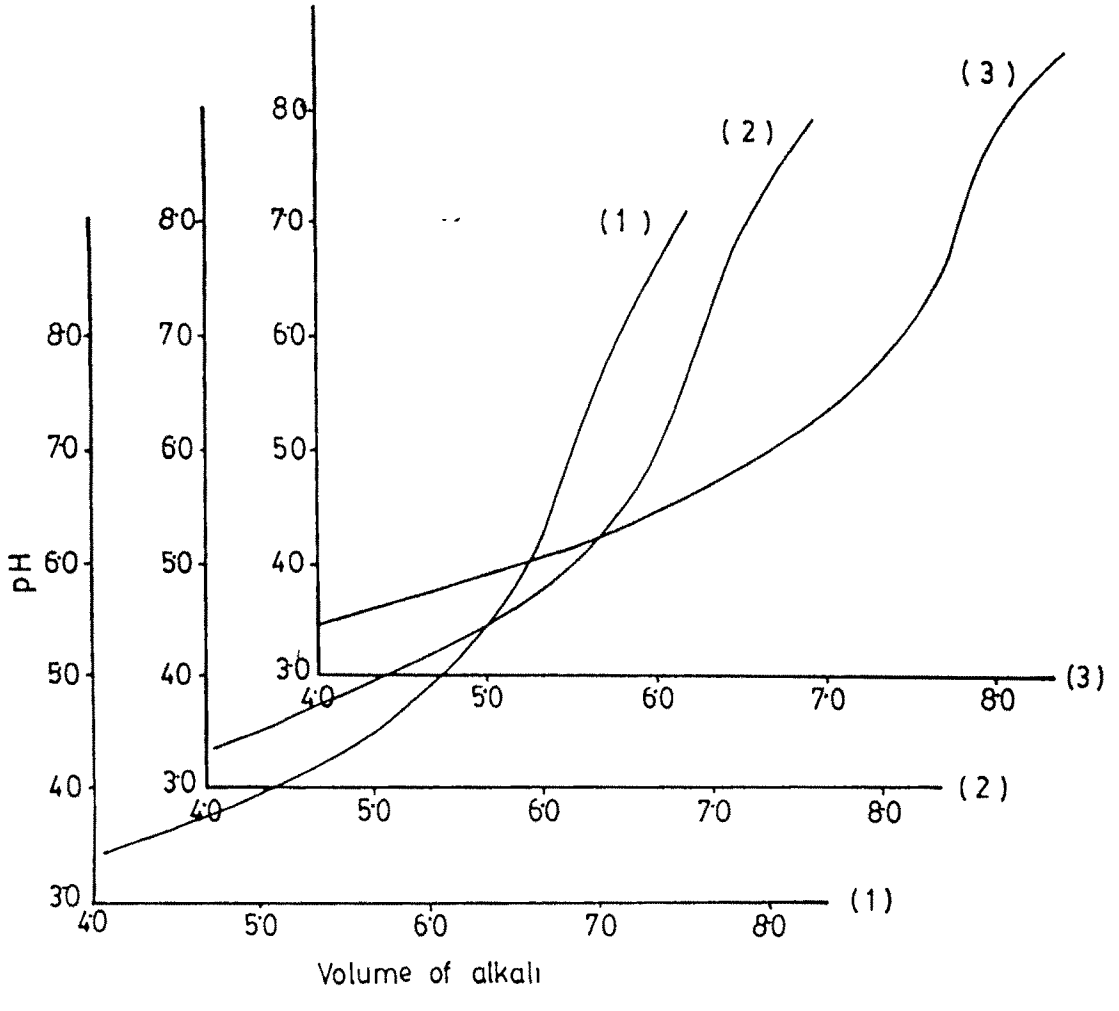
Ligand	pK_1^H	pK_2^H	pK_3^H	$\log K_{CuLH}^{Cu}$	$\log K_{CuL}^{Cu}$	$\log K_{CuL_2}^{CuL}$
Histidine(hist)	9.26	6.49	1.48	8.01	10.50	7.43
	(0.01)	(0.01)	(0.08)	(0.01)	(0.01)	(0.02)

Table 2B.3 : Formation constant of mixed-ligand complexes and $\Delta\log K$ in aqueous medium at I = 0.2M ($NaClO_4$) and 25°C with standard deviation ($\sigma\sim\beta$) in parentheses.

Complexes	$\text{Log}K^{Cu}_{CuALH}$	$\Delta\log K_{CuALH}$	$\log K^{Cu}_{CuAL}$	$\Delta\log K_{CuAL}$	$\log K^{CuA}_{CuAL}\text{-H}^{L}$
Cu-gg-hist.	20.40	+ 0.12	15.61	- 0.67	8.82
	(0.07)		(0.01)		(0.03)
Cu-ga-hist.	21.06	+ 0.72	16.14	- 0.20	8.57
	(0.02)		(0.01)		(0.02)
Cu-gl-hist.	20.77	+ 0.34	15.79	- 0.64	8.98
	(0.06)		(0.02)		(0.04)

Fig. 2B.1 Potentiometric titration curves of aqueous solutions containing metal ions, gg, ga or gl and hist (each 4.0 x 10^{-3} M)

(1) Cu^{2+} + gg + hist.
(2) Cu^{2+} + ga + hist.
(3) Cu^{2+} + gl + hist.



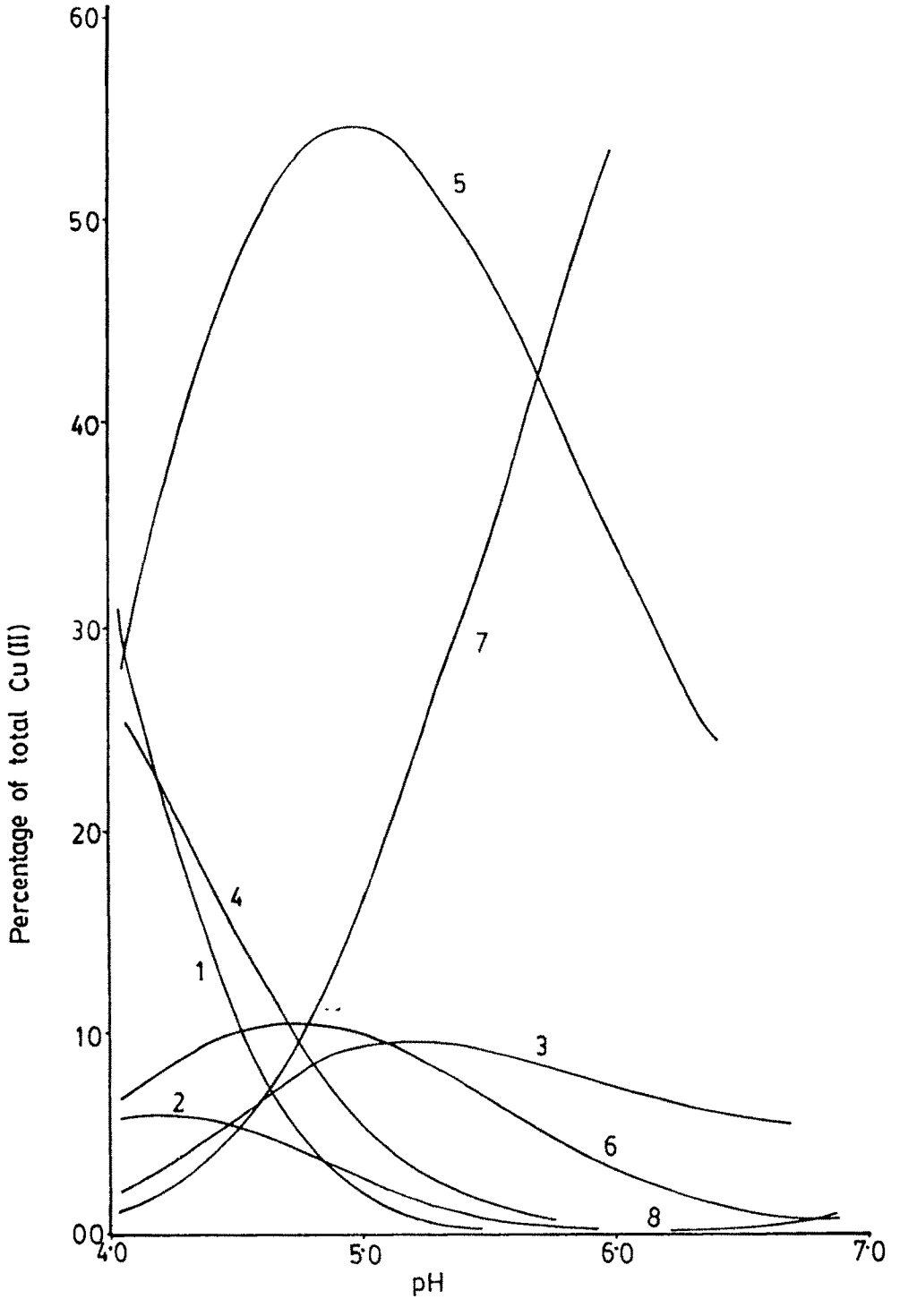


Fig. 2B.2 Species Distribution for the copper (11) - Glycylglycine (A) - Histidine (L) ternary system at Metal : A : L ratio of 1:1:1 ;

(1) Unbound copper(11), (2) [CuA], (3) [CuA_{-H}], (4) [CuLH], (5) [CuL) (6) [CuALH], (7) [CuAL] and (8) [$CuA_{-H}L$].

RESULTS AND DISCUSSIONS

Species distribution curves for the system copper(II) - glycylglycine-histidine (fig.2B.2) show the formation of three different ternary complexes at different pH, in addition to the binary complexes. The species CuALH starts forming at a pH ~4.0 and attains a maxium concentration at pH~ 5.0. The ternary complex CuAL is formed (about 10%) at pH ~ 5.0 and its concentration increases steadily with the increase in pH. The concentration of the deprotonated ternary complex is generally low. It is observed that this species starts forming at a pH ~ 6 and its concentration increases with the increase of pH.

Formation and Stability of CuALH Species :

In the CuALH species, which is formed at lower pH, coordination of histidine occurs through amino nitrogen and oxygen of the carboxylate group and the imidazole nitrogen remains free and protonated. Coordination of the dipeptide takes place through amino nitrogen and oxygen of the C=O group, whereas the carboxylate moiety of the dipeptide remains uncoordinated. The equilibrium constant for the above species is given by :

$$Cu + A + LH \rightleftharpoons CuALH \qquad (2B.1)$$

$$K^{Cu}_{CuALH} = \frac{[CuALH]}{[Cu][A](LH]} \qquad (2B.2)$$

$$\log K^{CuA}_{CuALH} = \log K^{Cu}_{CuALH} - \log K^{Cu}_{CuA} \qquad (2B.3)$$

$$\Delta \log K_{CuALH} = \log K^{CuA}_{CuALH} - \log K^{Cu}_{CuLH} \qquad (2B.4)$$

Δ log K for the ternary complex CuALH, as shown by equation 2B.4, for all the systems under study, was found to be positive, indicating thereby that this species is stabilised. This can be explained by considering that protonated histidine is neutral and does not repel the incoming dipeptide anion. Further, there is an electrostatic interaction between the protonated positively charged imidazole moiety of histidine and negatively charged carboxylate group of dipeptide (fig.2B.3). These two effects enhance the stability of the ternary species.

Formation and Stability of CuAL Species :

In the case of CuAL species, histidine is coordinated through amino nitrogen and imidazole nitrogen, its carboxylate group remaining free, and the dipeptide has the coordination sites NH_2 and peptide C=O. The equilibrium constant of the system can be represented by the following equations:

$$Cu + A + L \rightleftharpoons CuAL \qquad (2B.5)$$

$$K^{Cu}_{CuAL} = \frac{[CuAL]}{[Cu][A][L]} \qquad (2B.6)$$

$$\log K^{CuA}_{CuAL} = \log K^{Cu}_{CuAL} - \log K^{Cu}_{CuA} \qquad (2B.7)$$

$$\Delta \log K_{CuAL} = \log K^{CuA}_{CuAL} - \log K^{Cu}_{CuL} \qquad (2B.8)$$

The negative charge on free carboxylate group of histidine exerts a repulsive force on the incoming dipeptide monoanion (fig.2B.4). Hence for the CuAL species Δ log K was found to be negative for all the systems under study.

Formation and Stability of $CuA_{-H}L$ Species :

In case of the ternary $CuA_{-H}L$ complex, the ligand A_{-H} is deprotonated dipeptide, coordinated to copper(II) through amino nitrogen, deprotonated peptide nitrogen, and the carboxylate oxygen. Coordination of histidine is through amino nitrogen and imidazole nitrogen. For this species, only the protonation constant of the amide proton can be determined and can be shown by the equation.

$$CuA_{-H}L + H \rightleftharpoons CuAL \qquad (2B.9)$$

$$K^{CuA_{-H}L}_{CuAL} = \frac{[CuAL]}{[CuA_{-H}L][H]} \qquad (2B.10)$$

The deprotonation of the dipeptide A in the ternary complex $CuA_{-H}L$ can be compared with the deprotonation in the binary complex CuA_{-H}. It is observed that the deprotonation of the dipetide in the ternary complex is very much reduced and takes place at a much higher pH as compared to its deprotonation in the binary complex, which takes place at a lower pH. This can be explained by the fact that the dipeptide on deprotonation forms a dianion with one negative charge on the carboxylate group and another on the deprotonated peptide nitrogen. There is a strong repulsion between the dipeptide dianion and histidine monoanion during formation of $CuA_{-H}L$ species. This inhibits the coordination of the dipeptide through the peptide N and hence reduces the deprotonation. Secondly, there is destabillization of the ternary copper(II) complex with the tridentate A_{-H} ligand, due to Jahn - Teller effect (fig.2B.5) as explained in Chapter IIA.

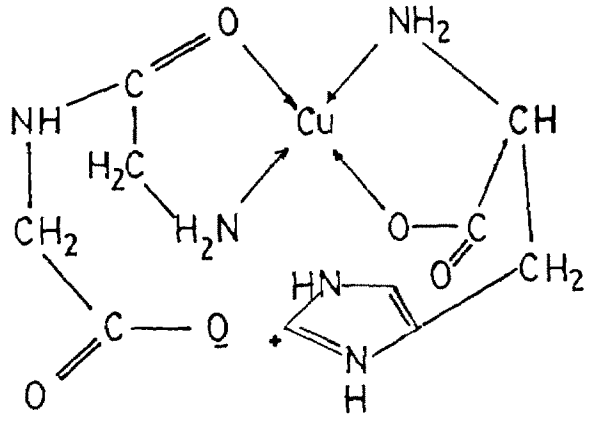


Fig. 2B·3

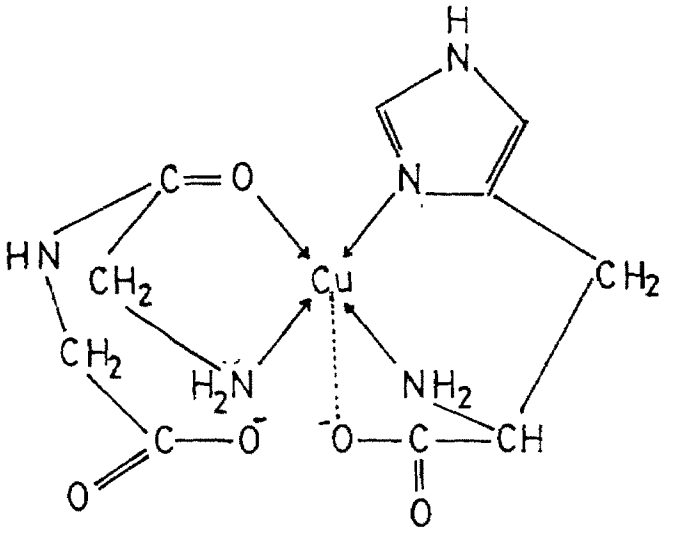


Fig. 2B·4

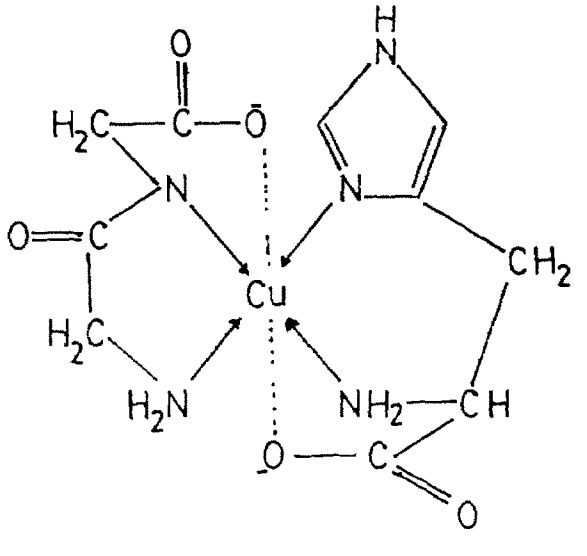


Fig. 2B·5

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