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The interaction of copper(II) and glycyl-L-histidyl-L-lysine, a growth-modulating tripeptide from plasma

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The interaction between Cu(II) and the growth-modulating tripeptide glycyl-L-histidyl-L-lysine in the presence and absence of L-histidine was investigated by potentiometric titration and visible-absorption spectrophotometry at 25°C in 0.15M-NaCl. Analyses of the results in the pH range 3.5–10.6 indicated the presence of multiple species in solution in the binary system and extensive amounts of the ternary complexes in the ternary system. The species distribution and the stability constants, as well as the visible-absorption spectra of the species, were evaluated. The combined results were used to propose the structure of some of the complexes. The influence of the ε-amino group of the peptide in the enhancement of the stability constants was reflected prominently when compared with those complexes formed by either glycyl-L-histidine or glycyl-L-histidylglycine. The results obtained from the equilibrium-dialysis experiments showed that this tripeptide was able to compete with albumin for Cu(II) at pH 7.5 and 6°C. At equimolar concentrations of albumin and the peptide, about 42% of the Cu(II) was bound to the peptide. At the physiologically relevant concentrations of Cu(II), albumin, L-histidine and this peptide, about 6% of the Cu(II) was associated with the low-molecular-weight components. This distribution could be due to the binary as well as the ternary complexes. The possible physiological role of these complexes in the transportation of Cu(II) from blood to tissues is discussed.

Human plasma contains approx. 1 μg of Cu(II)/ml. Most of this (90–95%) is tightly bound to ferroxidase (caeruloplasmin) and is not exchangeable *in vivo*. The remainder (5–10%) is associated with albumin and low-molecular-weight components such as L-histidine. It is the latter fraction that is in rapid equilibrium and is considered to be physiologically important (Bearn & Kunkel, 1954; Sarkar & Kruck, 1966; Neumann & Sass-Kortsak, 1967; Sarkar & Wigfield, 1968; Lau & Sarkar, 1971). A tripeptide glycyl-L-histidyl-L-lysine has been co-isolated with copper and iron from human plasma in association with the albumin and α-globulin fractions at about 200 ng/ml (Pickart & Thaler, 1973, 1980; Schlesinger *et al.*, 1977; Pickart *et al.*, 1979). It acts synergistically with these transition metals to alter the growth and metabolism of cultured hepatoma cells and hepatocytes. Evidence also indicates that this peptide forms complexes with the transition metals before interaction with cells. Thus these complexes have been suggested to function in the delivery of metallic elements required for cellular growth and survival in forms that are both non-toxic and utilizable by the cells. Since the peptide is isolated in highest concentration from the albumin-rich fraction of plasma, it has also been inferred to form a ternary complex with Cu(II) and albumin, as does L-histidine (Sarkar & Wigfield, 1968; Lau & Sarkar, 1971), as well as to participate in the regulation of Cu(II). In view of the potential physiological role of this peptide, detailed studies of the interaction between Cu(II) and the peptide with and without the presence of L-histidine have now been performed. Equilibrium studies were also undertaken to obtain information as to its affinity for Cu(II) as compared with albumin and the equilibrium distribution of Cu(II) in solution containing physiologically relevant concentrations of the peptide, albumin, Cu(II) and L-histidine. The presence of the multiple species

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in both binary and ternary complexes as well as their possible role in the transportation of Cu(II) from plasma to tissues are discussed.

Experimental

Materials

Crystalline human serum albumin obtained from Hoechst Pharmaceutical Co. (c/o Calbiochem-Behring Corp., La Jolla, CA, U.S.A.) was used without further purification. The radioisotope $^{67}Cu(II)$ was obtained from Atomic Energy of Canada (Toronto, Ont., Canada). Dialysis membrane was from Union Carbide (Toronto, Ont., Canada). Potassium hydrogen phthalate was purchased from the National Bureau of Standards (Washington, DC, U.S.A.). L-Histidine (free base) was obtained from Nutritional Biochemicals (Cleveland, OH, U.S.A.). The acetate form of glycyl-L-histidyl-L-lysine (Gly-His-Lys) was obtained from Bachem A.-G. (Bubendorf, Switzerland). This peptide was converted into the hydrochloride form before use by dissolving it in water and adjusting the pH of the solution to about 2.0, and was then freeze-dried. The peptide and L-histidine stock solutions were prepared in water and 30mM-HCl respectively and stored in the cold and dark. All other reagents were of analytical grade.

Potentiometric titration

The titrations were performed on a Radiometer automatic titration assembly thermostatically controlled at 25°C. The electrodes were calibrated against standard National Bureau of Standards buffers. NaOH, prepared carbonate-free and kept under argon atmosphere, was standardized against primary standard potassium hydrogen phthalate. Two stock solutions of $CuCl_2$ were prepared in 1mM-HCl (binary system) and 30mM-HCl (ternary system) and standardized complexometrically. All the solutions contained 0.15M-NaCl and known amounts of HCl to lower the starting pH below that of metal-binding. With the Cu(II)–peptide binary system, the solutions were titrated with 0.0983M-NaOH in the presence and absence of Cu(II) from pH2.4 to pH11.6, whereas in the Cu(II)–peptide–L-histidine ternary system the solutions were titrated with 1.021M-NaOH from pH1.6 to pH11.6. All the calculations were performed by a sequential use of three programs on a GE-400 computer.

Spectrophotometry

The visible-absorption spectra of the Cu(II) complexes were recorded on a Beckman Acta model MVI spectrophotometer as a function of pH in 0.15M-NaCl solutions at 25°C.

Equilibrium dialysis

The competition for Cu(II) between the tripeptide and albumin was conducted in 0.1M-*N*-ethylmorpholine/HCl buffer, pH7.53 and *I*0.16, at 6°C by the equilibrium-dialysis technique (Lau & Sarkar, 1971). One half-cell contained the stock solution of Cu(II)–albumin (0.145mM) mixed with $^{67}CuCl_2$, and the other half-cell contained various amounts of the peptide. The molar ratio of the peptide to Cu(II)–albumin ranged from 1.03:1.00 to 103:1.0. Radioactivity of $^{67}Cu(II)$ was measured before and after dialysis. The distribution of Cu(II) in the presence of their typical physiological concentrations of albumin, exchangeable amount of Cu(II), L-histidine and Gly-His-Lys in serum (Neumann & Sass-Kortsak, 1967; Pickart & Thaler, 1973; Schlesinger *et al.*, 1977) was also measured by the same method.

Results

Determination of species distribution and stability constants

The general equilibrium involving metal ion M, proton H, ligands A and B can be represented as:

$$pM + qH + rA + sB \rightleftharpoons M_pH_qA_rB_s \qquad (1)$$

where p, q, r and s are the stoicheiometric quantities of M, H, A and B respectively. The stabilities of the species formed are expressed as the stoicheiometric equilibrium constants β_{pqrs} in terms of concentrations at constant ionic strength, temperature and pressure:

$$\beta_{pqrs} = \frac{M_pH_qA_rB_s}{m^p h^q a^r b^s} \qquad (2)$$

where m, h, a and b are the concentrations of free metal ion, free H^+ ion and completely deprotonated ligands A and B respectively. The titration results were obtained with solutions containing different concentrations, C_M, C_H, C_A and C_B. The mathematical analyses of the titration data were performed by the sequential use of three computer programs, PLOT-3, GUESS-3 and LEASK-4 (written in BASIC language). The program PLOT-3 takes the numerical titration data and calculates the proton-liberation terms $\delta H^+/\delta C_M$, $\delta H^+/\delta C_A$ and $\delta H^+/\delta C_B$ and the amounts of the free metal ion and ligands A and B at each selected pH value. The program GUESS-3, using data from PLOT-3, sets up a matrix of the terms $m^p h^q a^r b^s$ for each proposed species. This matrix then serves as an input to the program LEASK-4, which uses an iterative least-squares minimization procedure to calculate the stability constants β_{pqrs}. A detailed account of the data processing has been previously reported

Table 1. *Sample composition of the binary systems M–H–A [M = Cu(II), A = glycyl-L-histidyl-L-lysine] titrated in 0.15 M-NaCl at 25°C*

Sample no.	Total amounts (μmol) Metal	Ligand A
Proton–ligand A system		
1	—	11.18
2	—	22.36
3	—	33.54
4	—	44.72
Metal–ligand A system		
Metal-concentration variation		
5	5.00	33.54
6	7.50	33.54
7	10.00	33.54
8	12.50	33.54
Ligand A-concentration variation		
9	7.50	11.18
10	7.50	22.36
11	7.50	33.54
12	7.50	44.72

(Sarkar & Kruck, 1973; Sarkar, 1977; Laurie *et al.*, 1979; Iyer *et al.*, 1978).

Proton-glycyl-L-histidyl-L-lysine system

Solutions 1–4 in Table 1 were titrated. The first and fourth protonation equilibria were sufficiently well separated from the other two to be treated with the Henderson–Hasselbach equation. The two overlapping equilibria in the pH 6–8 region were processed by programs outlined above to obtain the constants β_{0210} and β_{0310} of the species H_2A and H_3A respectively. These values, given as pK_a values for the successive protonated species, are listed in Table 3.

Cu(II)–proton–glycyl-L-histidyl-L-lysine system

In the metal-concentration-variation experiments solutions 5–8 (Table 1) were titrated, and in the ligand-concentration-variation experiments solutions 9–12 (Table 1) were titrated. The data were processed by program PLOT-3 to yield $\delta H^+/\delta C_M$ (Fig. 1, curve *A*) and $\delta H^+/\delta C_A$ (Fig. 1, curve *B*) as a function of pH. For the species selection of the complexes formed in the metal–ligand system, the following values for *p*, *q* and *r* were used: $p = 1$; $q = +3, +2, +1, 0, -1, -2$; $r = 1, 2$. The intermediate data as a function of pH were further processed by programs GUESS-3 and LEASK-4. The calculations indicated that the complex species MA, $MH_{-1}A$, $MH_{-2}A$, MH_2A_2, MHA_2, MA_2, $MH_{-1}A_2$ and $MH_{-2}A_2$ were required to give a minimum-error solution. Their stability constants

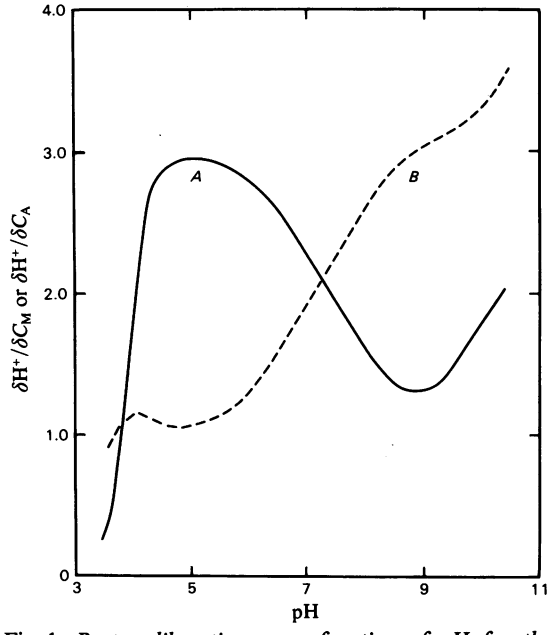

Fig. 1. *Proton liberation as a function of pH for the system Cu(II)-glycyl-L-histidyl-L-lysine*
Curve *A* (——), $\delta H^+/\delta C_M$; curve *B* (– – – –), $\delta H^+/\delta C_A$. For details see the text.

expressed as $\log\beta_{pqrs}$ are listed in Table 3, and the computed species distribution as a function of pH is shown in Fig. 2.

Cu(II)–proton–glycyl-L-histidyl-L-lysine–L-histidine system

In the metal-concentration-variation experiments solutions 1–4 in Table 2 were titrated, and in the ligand-concentration-variation titrations solutions 5–8 for ligand A and solutions 9–12 for ligand B were used (Table 2). The data were processed likewise to yield $\delta H^+/\delta C_M$ (Fig. 3, curve *A*), $\delta H^+/\delta C_A$ (Fig. 3, curve *B*) and $\delta H^+/\delta C_B$ (Fig. 3, curve *C*) as a function of pH. For the species selection of the complexes formed in the system, the following values for *p*, *q*, *r* and *s* were used: $p = 1$; $q = +2, +1, 0, -1, -2$; $r = 1, 2$; $s = 1, 2$. To solve for stability constants for the ternary complexes, all previously determined stability constants from the binary systems were kept constant and only those of the ternary species $M_pH_qA_rB_s$ were treated as variables (Kruck & Sarkar, 1973*a*). The minimum-error solution for the ternary system permitted the presence of four ternary complexes, MH_2AB, MHAB, MAB and $MH_{-1}AB$. Their stability constants are included in Table 3, and the species distribution as a function of pH is shown in Fig. 4.

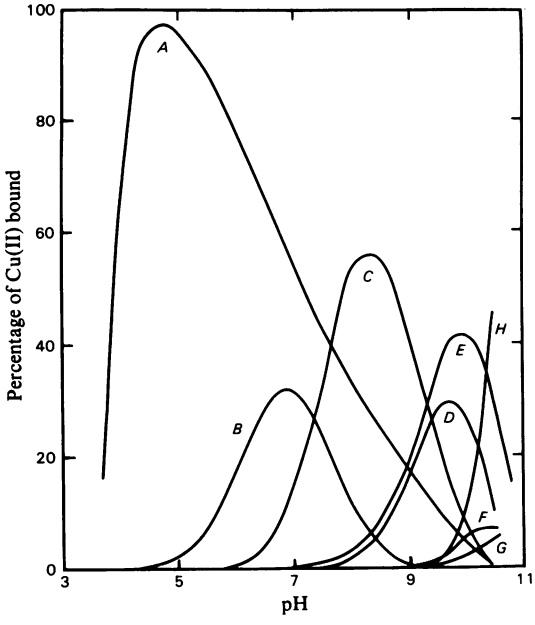

Fig. 2. *Computed species distribution as a function of pH for the system Cu(II)–glycyl-L-histidyl-L-lysine*
Curve *A*, MA; curve *B*, MH_2A_2; curve *C*, MHA_2; curve *D*, MA_2; curve *E*, $MH_{-1}A$; curve *F*, $MH_{-1}A_2$; curve *G*, $MH_{-2}A$; curve *H*, $MH_{-2}A_2$. For details see the text.

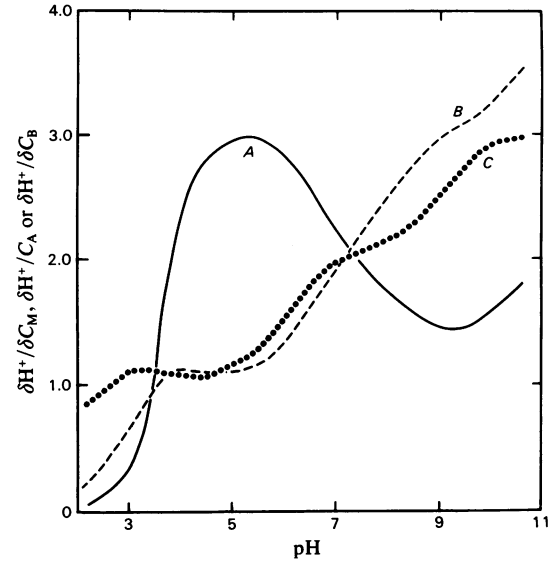

Fig. 3. *Proton liberation as a function of pH for the system Cu(II)–glycyl-L-histidyl-L-lysine–L-histidine*
Curve *A* (——), $\delta H^+/\delta C_M$; curve *B* (– – – –), $\delta H^+/\delta C_A$; curve *C* (·····), $\delta H^+/\delta C_B$. For details see the text.

Table 2. *Sample composition of the ternary systems M–H–A–B [M = Cu(II), A = glycyl-L-histidyl-L-lysine, B = L-histidine] titrated in 0.15 M-NaCl at 25°C*

Sample no.	Total amounts (μmol) Metal	Ligand A	Ligand B
Metal-concentration variation			
1	5.00	27.66	24.74
2	10.00	27.66	24.74
3	20.00	27.66	24.74
4	25.00	27.66	24.74
Ligand A-concentration variation			
5	10.00	9.22	24.74
6	10.00	18.44	24.74
7	10.00	27.66	24.74
8	10.00	36.88	24.74
Ligand B-concentration variation			
9	10.00	27.66	9.90
10	10.00	27.66	19.79
11	10.00	27.66	24.74
12	10.00	27.66	39.58

Visible-absorption spectra

In the binary system, the spectrum of a solution containing an M:A molar ratio of 1.00:1.58 and 0.15 M-NaCl was recorded at selected pH values. The total Cu(II) concentration was 3.85 mM. Representative spectra are shown in Fig. 5. The spectrum obtained at pH 4.31 was contributed from the single species MA, which corresponded to about 93% of the total Cu(II) in the solution. The spectral characteristics of the species MA ($\lambda_{max.}$ 610 nm, $\varepsilon_{max.}$ 61 $M^{-1}\cdot cm^{-1}$), together with the spectral data for various pH values, were processed by the method described previously to yield the calculated spectra for individual species $M_pH_qA_r$ (Kruck & Sarkar, 1975). The results are shown in Table 4. In the ternary system, the spectrum of a solution containing M:A:B proportions of 1.00:1.68:1.60 and 0.15 M-NaCl was also recorded as a function of pH (Fig. 6). The total Cu(II) concentration was 4.0 mM. The spectral data at various pH values together with the predetermined spectral characteristics of the binary species (Kruck & Sarkar, 1973*b*) were likewise processed to give the calculated spectra for individual species $M_pH_qA_rB_s$. Their spectral characteristics are included in Table 4.

Equilibrium-dialysis studies

The distribution of ^{67}Cu(II) radioactivity at equilibrium indicated that the peptide Gly-His-Lys was able to compete with albumin for Cu(II) (as

Table 3. *pK_a and log(stability constants) (β_{pqrs}) of the complex species $M_pH_qA_rB_s$ [M = Cu(II), A = peptides, B = L-histidine] in 0.15 M-NaCl at 25°C*

For details see the text.

					$\log\beta_{pqrs}$			
p	*q*	*r*	*s*	pK_a	Gly-His-Lys	Gly-His*	Gly-His-Gly*	Gly-Gly-His†
0	4	1	0	2.91				
0	3	1	0	6.53				
0	2	1	0	7.93				
0	1	1	0	10.44				
1	0	1	0		16.44	8.68	8.52	7.55
1	−1	1	0		7.48	4.54	5.32	2.68
1	−2	1	0		−3.74	−4.94	−3.69	−1.92
1	2	2	0		38.18			25.81
1	1	2	0		30.83	20.45		20.64
1	0	2	0		21.43	15.41	15.78	16.68
1	−1	2	0		10.76	7.68	8.41	9.73
1	−2	2	0		1.08			1.43
1	2	1	1		34.45			
1	1	1	1		29.02			
1	0	1	1		21.09			
1	−1	1	1		11.45			

* Agarwal & Perrin (1975).
† Lau & Sarkar (1981).

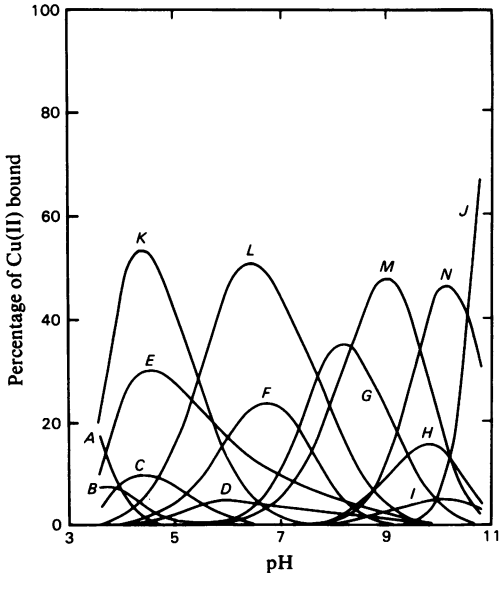

Fig. 4. *Computed species distribution as a function of pH for the system Cu(II)–glycyl-L-histidyl-L-lysine–L-histidine*

Curve *A*, MHB; curve *B*, MB; curve *C*, MHB_2; curve *D*, MB_2; curve *E*, MA; curve *F*, MH_2A_2; curve *G*, MHA_2; curve *H*, MA_2; curve *I*, $MH_{-1}A$; curve *J*, $MH_{-2}A_2$; curve *K*, MH_2AB; curve *L*, MHAB; curve *M*, MAB; curve *N*, $MH_{-1}AB$. For details see the text.

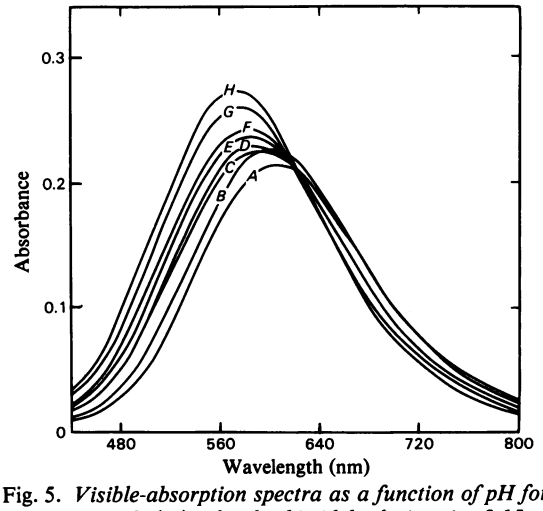

Fig. 5. *Visible-absorption spectra as a function of pH for the system Cu(II)–glycyl-L-histidyl-L-lysine in 0.15 M-NaCl at 25°C*

$[Cu(II)]_t$ = 3.85 mM, $[A]_t$ = 6.07 mM, light-path = 1 cm. Curve *A*, pH 4.31; curve *B*, pH 5.82; curve *C*, pH 7.20; curve *D*, pH 8.06; curve *E*, pH 9.10; curve *F*, pH 9.44; curve *G*, pH 9.94; curve *H*, 10.42. For further details see the text.

shown in Fig. 7). At equimolar concentrations, more than 40% of the Cu(II) was bound to the peptide. Thus this peptide shows Cu(II)-binding affinity

Table 4. *Spectral characteristics of the complex species* $M_pH_qA_rB_s$ [*M* = *Cu(II)*, *A* = *glycyl-L-histidyl-L-lysine*, *B* = *L-histidine*] *in 0.15 M-NaCl at 25°C*
For details see the text.

p	*q*	*r*	*s*	$\lambda_{max.}$ (nm)	$\varepsilon_{max.}$ ($M^{-1} \cdot cm^{-1}$)
1	0	1	0	610	61
1	−1	1	0	580	80
1	−2	1	0	560	127
1	2	2	0	590	65
1	1	2	0	580	57
1	0	2	0	595	48
1	−1	2	0	570	90
1	−2	2	0	550	98
1	2	1	1	610	71
1	1	1	1	580	62
1	0	1	1	590	84
1	−1	1	1	580	89

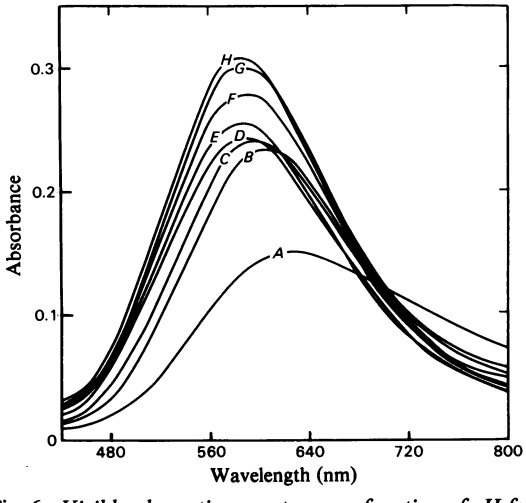

Fig. 6. *Visible-absorption spectra as a function of pH for the system Cu(II)–glycyl-L-histidyl-L-lysine–L-histidine in 0.15 M-NaCl at 25°C*
$[Cu(II)]_t$ = 4.00 mM, $[A]_t$ = 6.71 mM, $[B]_t$ = 6.40 mM, light-path = 1 cm. Curve *A*, pH 3.95; curve *B*, pH 5.17; curve *C*, pH 6.06; curve *D*, pH 6.87; curve *E*, pH 7.81; curve *F*, pH 8.62; curve *G*, pH 9.49; curve *H*, pH 10.20. For further details see the text.

comparable with that of the peptides glycylglycyl-L-histidine (Gly-Gly-His) and L-aspartyl-L-alanyl-L-histidine *N*-methylamide (Asp-Ala-His-NHMe) (Lau *et al.*, 1974; Iyer *et al.*, 1978). With more physiologically relevant concentrations of albumin, Cu(II), L-histidine and Gly-His-Lys as found in plasma, results obtained were very similar to those with native serum (Neumann & Sass-Kortsak, 1967;

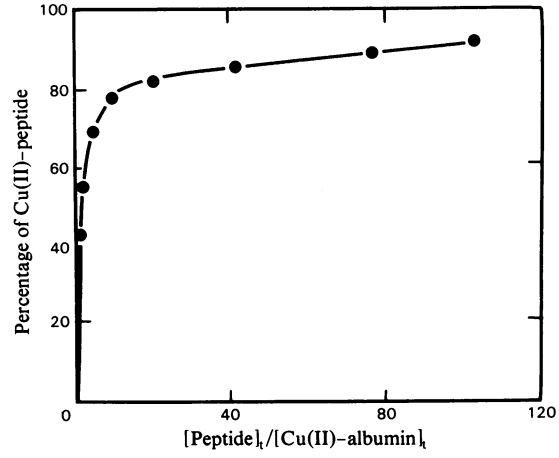

Fig. 7. *Formation of low-molecular-weight species in the system albumin–Cu(II)–glycyl-L-histidyl-L-lysine at pH 7.53 and 10.16 at 6°C*
$[Cu(II)\text{–albumin}]_t$ = 0.145 mM. For further details see the text.

Pickart & Thaler, 1973; Schlesinger *et al.*, 1977), namely that about 6% of the Cu(II) was found to be associated with the low-molecular weight components.

Discussion

The peptide Gly-His-Lys has four titratable protons in the pH range studied (2.4–11.6). They are attributed to the carboxy group, the imidazole group and both α- and ε-amino groups, with pK_a values of 2.91, 6.53, 7.93 and 10.44 respectively. In the binary system, the titration results for minimum-error solution indicate the presence of the following species: MA, $MH_{-1}A$, $MH_{-2}A$, MH_2A_2, MHA_2, MA_2, $MH_{-1}A_2$ and $NH_{-2}A_2$. The proton displacement profile shows the peptide starting to bind Cu(II) at about pH 3, and liberating a maximum of 3 protons in a range of less than 2 pH units. This rapid displacement corresponds to the formation of the major species MA in this pH region. Since the carboxy group is already titrated, the three protons can be attributed to those from either the imidazole group and both α- and ε-amino groups, or the imidazole group, amino group and the peptide backbone nitrogen atom. A structure involving one carboxy group, one imidazole group and two other types of nitrogen atoms co-ordinating to the central Cu(II) ion in a square-planar configuration is consistent with the observed maximal visible absorption of about 610 nm (Bryce & Gurd, 1966). An X-ray-crystallographic study of a Cu(II)–Gly-His-Lys complex showed the existence of a tridentate

bonding structure involving the imidazole group, the α-amino group and the peptide backbone nitrogen atom (Pickart *et al.*, 1980), as was the case of glycyl-L-histidine (Gly-His) (Blount *et al.*, 1967). However, the much higher value of the log (stability constant) of species MA ($\log_{101} = 16.44$) as compared with the corresponding Cu(II) complexes formed by Gly-His and glycyl-L-histidylglycine (Gly-His-Gly) ($\log_{101} = 8.68$ and 8.52 respectively at 37°C in 0.15M-KNO_3; Table 3) [the effects of temperature and background electrolyte do not seem to cause such a dramatic effect in the log (stability constants) of the species detected in many of the metal–ligand systems] (Agarwal & Perrin, 1975) may suggest the involvement of the ε-amino group in the co-ordination of Cu(II) ion in solution. The difference in the structure of the complex species determined by X-ray analysis and that obtained by solution studies may result from the different experimental conditions. By the very nature of our species analyses, one obtains a detailed description of simultaneously existing multiple species as a function of pH. This is not the case with the X-ray studies. It is difficult to ascertain which forms of the complex crystallized out from a multispecies system, particularly if they had a similar proton balance. Hence it is possible that we are referring to two different species. Further studies by n.m.r. utilizing a similar approach to that followed with Cu(II)–Asp-Ala-His-NHMe (Laussac & Sarkar, 1980) might provide clearer indication of the structure of the species MA.

The stepwise ionization of the species MA showed successive pK values of 8.96 and 11.22 in forming species $MH_{-1}A$ and $MH_{-2}A$. Together with the spectral results of lowering the maximal absorption to 580 and 560nm respectively, these ionizations could be ascribed to the consecutive involvement of the peptide nitrogen atoms in the formation of $MH_{-1}A$ and $MH_{-2}A$. However, the co-operative action of the metal-assisted ionizations does not seem to be very pronounced here, as has been observed with some peptides, such as Gly-Gly-His (Bryce *et al.*, 1965; Lau & Sarkar, 1981).

Extensive amounts of bis-complexes were detected in the system at neutral pH and above (Fig. 2). The higher values of the stability constants were also found for the species MHA_2, MA_2 and $MH_{-1}A_2$ as compared with the corresponding values for both peptides Gly-His and Gly-His-Gly. This observation again reflects the influence of the additional ε-amino group on the stability (Table 3). Among these bis-complexes, only MH_2A_2 and MHA_2 are of physiological significance. Analyses of the proton-displacement data and the equilibrium distribution in the region with no extensive overlap would provide an estimation of the number of protons liberated for certain species. This information, with the aid of the known pK_a values of the functional groups as well as the spectral results, could then lead to the proposed structure for these species. For instance, at pH 6 the estimated contribution of proton from the species MA would be about 2.20. The difference from the observed value of 2.85 protons is due to the presence of 20% of the species MH_2A_2. Thus the estimated value for MH_2A_2 would be about 3.25 protons, which most probably arose from either the amino and imidazole groups or the peptide nitrogen atom and imidazole group of each molecule of the peptide. The calculated maximal absorption of 590nm for MH_2A_2 is in accord with this contention. Similar consideration of the results would yield the number of protons liberated at pH 7.5 for the species MHA_2 as 2.77. As the calculated maximal absorption of 580nm was obtained for MHA_2, its structure could be proposed as involving the imidazole group, two α-amino groups and either one ε-amino group or a peptide nitrogen atom to coordinate the central Cu(II) ion.

The results obtained from equilibrium dialysis indicate that the tripeptide Gly-His-Lys can remove Cu(II) from the Cu(II)–albumin complex very effectively at neutral pH. At equimolar concentrations 42% of the Cu(II) was bound to Gly-His-Lys (Fig. 7), compared with about 12% and 44% in the case of L-histidine and Gly-Gly-His respectively (Lau & Sarkar, 1971; Lau *et al.*, 1974). The efficiency of Gly-His-Lys over L-histidine is also reflected in the higher stability of the Cu(II)–peptide complexes. It is noteworthy that Gly-His-Lys has an affinity for Cu(II) comparable with that of Gly-Gly-His, which was designed to mimic the Cu(II)-transport site of albumin (Lau *et al.*, 1974). Since Gly-His-Lys has been isolated in highest concentration from the albumin-rich fraction of plasma, the possibility of forming a ternary complex between Gly-His-Lys, Cu(II) and albumin cannot be ruled out. The physiological role of the ternary complex cannot be discerned at the present time.

In the ternary system containing Gly-His-Lys, Cu(II) and L-histidine, analyses of the titration results indicated the presence of four ternary complexes, namely MH_2AB, $MHAB$, MAB and $MH_{-1}AB$, in the pH region studied, in addition to several binary species. Owing to the extensive overlap of the different species, it is difficult to obtain useful information about the structural features of these ternary complexes from the combined results of the proton-displacement data, the species distribution and the computed visible-absorption spectra. However, the fact of detection of about 45% of the ternary complexes at pH 7.5 suggests the possible significance of their presence in solution. Under the condition of having physiologically relevant concentrations of Cu(II), albumin, Gly-His-Lys and L-histidine, the equilibrium distribution of Cu(II)

showed a result similar to that obtained with native serum. Only about 6% of the Cu(II) was associated with the low-molecular-weight components. On the basis of the equilibrium distribution from the titration work, as well as the earlier observation that the Cu(II) complexes of this peptide are normally present in the human blood circulation, this 6% of Cu(II) could be distributed among both the binary and the ternary complexes. Since Gly-His-Lys is capable of promoting the cell growth, especially in the presence of transition metals such as Cu(II) (Pickart *et al.*, 1979, 1980; Pickart & Thaler, 1980), it has been suggested to participate in the cell-surface mediated transport of Cu(II) from blood to tissues. The peptide seems to act in the delivery of Cu(II) ion required for cellular growth and survival in forms that are both non-toxic and utilizable by the cells. In view of our findings, it is difficult to postulate whether binary or ternary form of the complex is responsible for the transportation of Cu(II). It is also uncertain which complex species is involved in the transport process and what effect the charge, stability and configuration of that particular species might have on the transport phenomenon. While maintaining the simultaneously existing equilibria of the Cu(II) complexes of albumin, Gly-His-Lys and amino acids in plasma, the conformation of Cu(II)–Gly-His-Lys and/or L-histidine–Cu(II)–Gly-His-Lys complexes could be such as to facilitate its initial interaction with the cell surface and then the delivery of Cu(II) across the membrane. Thus investigation of the interaction of the Cu(II) complexes of Gly-His-Lys with plasma membrane should help in delineating the transport mechanism.

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