Copper(II) and Zinc(II) Complexes of Glycylglycyl-L-histidine and Derivatives

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Computer-assisted analysis of pH-titration data has afforded equilibrium constants at 37 °C and I = 0.15 mol dm^{-3} (K[NO_3]) for copper(II) and zinc(II) complexes of glycylglycyl-L-histidine (Gly-Gly-His), Gly-Gly-His-Gly-Gly, their alkyl esters, and their benzyloxycarbonyl derivatives. The types of complexes and their equilibrium values are similar to those formed by Gly-His. Binuclear complexes are major components of the systems containing Zn^{II}. The copper peptide complexes are reasonable models for the binding of Cu^{II} by albumin, but the disparity between constants for zinc–peptide and –albumin complexes indicates that a different binding site is involved.

THE terminal portion Asp-Ala-His of bovine serum albumin binds copper(II) ions strongly in a square-planar complex involving the amino-group of the aspartic acid (Asp) residue, an imidazole nitrogen of the histidine (His), and two intermediate peptide nitrogens (Ala = alanine).[1-3] The peptides Gly-Gly-His and Gly-Gly-His-Gly-Gly (Gly = glycine) and their esters contain the same potential metal-binding sites and hence might be expected to serve as model compounds in the interpretation of copper–albumin interactions. Zinc ions are also bound strongly by albumin but the co-ordination site has not been established. For this reason we have included these peptides in our quantitative study of the interaction of copper(II) and zinc(II) ions with histidine-containing peptides in aqueous solution. We have also compared the stability constants of these complexes with published[4-6] values for related species. Bryce *et al.*[7] determined pK_a values for proton loss for the alkalimetric titration of a 1 : 1 mixture of Cu^{2+} and Gly-Gly-His, and Lau *et al.*[8] made a more detailed study of equilibria in the Cu^{II}–Gly-Gly-His system by analytical potentiometry at 25 °C and I = 0.15 mol dm^{-3} NaCl. However, the latter workers used very dilute peptide solutions (*ca.* 10^{-4} mol dm^{-3}) so that only 1 : 1 complexes were detected, except in the presence of 6×10^{-3} mol dm^{-3} His when ternary histidinecopper(II)–peptide complexes were also formed.[8]

EXPERIMENTAL

Materials.—The peptides Gly-Gly-His, Gly-Gly-His-Gly-Gly, and their derivatives were synthesised from glycylglycine (Fluka, puriss.), L-histidine methyl ester dihydrochloride (Fluka puriss.), and benzyloxycarbonyl chloride (Fluka, pract.). Benzyloxycarbonylglycylglycylhistidine methyl ester (from benzyloxycarbonylglycylglycyl hydrazide[9,10] and histidine methyl ester by the method of Yokoyama *et al.*[11]) was purified by reprecipitation at *ca.* pH 8.5 from an acid solution of the ester, washed with water, and dried *in vacuo*. The ethyl ester of $PhCH_2OCO$-Gly-Gly-His-Gly-Gly was similarly synthesised from benzyloxycarbonylglycylglycylhistidinyl hydrazide[10] and glycylglycine ethyl ester. Hydrogenation during 4 h, over palladium black,[12] of the benzyloxycarbonyl peptide esters (0.01 mol) dissolved in methanol (50 cm^3) and 1 mol dm^{-3} HCl (20.5 cm^3) afforded the corresponding peptide ester dihydrochlorides which, after removal of the catalyst by filtration and evaporation of the solution to dryness *in vacuo*, were purified by precipitation with diethyl ether from alcoholic solution. The free peptides were obtained from the peptide ester dihydrochlorides (0.01 mol) by saponification with aqueous Na[OH] (31 cm^3, 1 mol dm^{-3} solution), at 15—20 °C during 0.5—1 h, followed by acidification with HCl and desalting over Dowex 50W–X4 ion-exchange resin in the H^+ form. After elution of the column with 1 mol dm^{-3} ammonium hydroxide and lyophilisation of the peptide-containing fractions, the peptides were recrystallised from aqueous methanol (for Gly-Gly-His) or ethanol (for Gly-Gly-His-Gly-Gly). Purity was checked by amino-acid analysis, by elemental analyses for C, H, and N, and by the presence of a single spot (in the Pauly reaction) following paper electrophoresis (2 000 V) for 60 min at pH 4.7. All the peptides and derivatives were dried over P_4O_{10} *in vacuo* before use.

Solutions of the peptides were prepared immediately before use in boiled-out glass-distilled water. All the other reagents were as previously described.[4,5]

Procedure.—The strengths of peptide solutions were checked by titration against standard copper(II) nitrate solution using a method similar to that for the standardisation of copper(II) solutions against ethylenediaminetetra-acetate (edta).[13] Potentiometric titrations at 37 °C and I = 0.15 mol dm^{-3} (K[NO_3]) and the resulting computations followed established procedures.[4,5] The (usually small) hydrogen- and hydroxide-ion concentrations, which were required for evaluating the stoicheiometry of titrations of ligand–metal-ion mixtures, were obtained from the hydrogen-ion activities as measured by a glass electrode by taking pK_w as 13.620 at 37 °C and assuming an activity coefficient of 0.80.[14] Ranges of metal-ion concentrations, ligand concentrations, and pH values used in the computation of

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TABLE 1

Summary of titrations used in obtaining the stability constants

Metal ion	10^3 Concentration / mol dm^{-3}	Ligand	10^3 Concentration / mol dm^{-3}	pH
Cu^{2+}	1.0—5.1	Gly-Gly-His	2.2—5.2	3.8—7.8
Zn^{2+}	1.0—5.0	Gly-Gly-His	1.0—5.4	5.8—7.9
Cu^{2+}	1.3—2.6	Gly-Gly-His-OMe	2.2—2.5	3.8—7.6
Zn^{2+}	1.0—1.9	Gly-Gly-His-OMe	2.4—2.4	5.4—7.0
Cu^{2+}	1.0—2.0	$PhCH_2OCO$-Gly-Gly-His-OMe	2.0—2.0	5.0—7.5
Zn^{2+}	1.9—2.5	$PhCH_2OCO$-Gly-Gly-His-OMe	2.8—4.1	5.7—6.5
Cu^{2+}	1.0—5.1	Gly-Gly-His-Gly-Gly	2.1—5.2	3.4—7.9
Zn^{2+}	1.0—5.0	Gly-Gly-His-Gly-Gly	2.0—5.2	5.4—7.4
Cu^{2+}	1.3—2.6	Gly-Gly-His-Gly-Gly-OEt	2.5—2.9	3.7—7.6
Zn^{2+}	1.3—2.5	Gly-Gly-His-Gly-Gly-OEt	2.5—2.8	6.1—7.4
Cu^{2+}	1.0—2.0	$PhCH_2OCO$-Gly-Gly-His-Gly-Gly-OEt	2.1—2.1	5.2—7.6

pK_a values of the ligands and stability constants of the metal complexes are summarised in Table 1. In order to study ternary complex formation by metal ion, histidine, and peptide, approximately equimolar concentrations (2.5×10^{-3}—5×10^{-3} mol dm^{-3}) of reagents were used for the titrations. In solutions containing equimolar concentrations of zinc and peptide, precipitation occurred at *ca.* pH 7.5 ±0.3. All the potentiometric–titration data are in Supplementary Publication No. SUP 21919 (25 pp., 1 microfiche).* Practical constants were refined from the titration data using the program SCOGS [15] on a Univac 1108 computer.

RESULTS AND DISCUSSION

By analogy with results for Gly-His [4] and His-His,[6] the pK_a values (for proton loss) of the peptides and derivatives in Table 2 are assigned as follows: 2.4—3.2, carboxyl group; 6.1—6.5, imidazolinium group; 7.4—7.5, protonated α-amino-group. Effects of benzyloxycarbonylation and esterification are consistent with these assignments.

Results with Gly-His suggested, and computer-based analysis of the titration data confirmed, that Cu^{II} and Gly-Gly-His form a series of 1 : 1 and 1 : 2 metal–ligand complexes in which the ligand varies from HL to $[L - 2H]^{3-}$, where L is the peptide anion. Stability constants given in Table 2 are consistently smaller for Gly-Gly-His complexes than for the corresponding Gly-His complexes, and these, in turn, are less than for His complexes. Thus log K for $Cu^{2+} + HL \rightleftharpoons [Cu(HL)]^{2+}$ is 4.19, 4.28, and 5.00, respectively, and for $[CuL]^+$ log K_1 is 7.04, 8.68, and 9.80. A similar decrease is observed for $[Cu(Gly\text{-}Gly\text{-}Gly)]^+$ (4.88) relative to $[Cu(Gly\text{-}Gly)]^+$ (5.34) and $[Cu(Gly)]$ (8.02).

The near agreement of log K_1 values for the 1 : 1 [Cu(Gly-Gly-His)]$^+$ complex (7.04) and the corresponding methyl ester complex (6.87) and the 1 : 1 [Cu(Gly-Gly-His-Gly-Gly)]$^+$ complex (6.83) and its ethyl ester complex (6.65) points to the non-involvement of the carboxyl group in the metal binding. Conversely, the much smaller value of log K_1 for the corresponding benzyloxycarbonyl ester complex indicates that the primary amino-group is one of the metal-bonding sites. We were unable to obtain stepwise pK_a values for the loss of successive protons from [Cu(Gly-Gly-His)]$^+$. Instead, two closely overlapping pK_a values with a mean of 4.57 at 37 °C were found. Similarly Bryce *et al.*[7] reported two pK_a values for [Cu(Gly-Gly-His)]$^+$ of 4.90 and 5.00 at 25 °C. Sundberg and Martin [16] ascribed such closeness of pK_a values to ' co-operative ionisation ', and explained its occurrence in [Cu(Gly-Gly-His)]$^+$ as follows; the amino-group at one end of Gly-Gly-His and the imidazole nitrogen at the other form the termini for three chelate rings only if both of the intervening peptide nitrogens are deprotonated. The observed pK_a values are thus due to the copper(II)-ion-promoted ionisation of peptide hydrogens.[16] In the resulting stable complex (conditional log K 12.3 at pH 7.4) the Cu^{2+} is co-ordinated to the amino-nitrogen, the two peptide nitrogens, and an imidazole nitrogen atom.[16] A similar square-planar structure has been established by *X*-ray studies of [Cu{Gly-Gly-His(NHMe)}]$^+$ crystals grown [17] at physiological pH and of [Cu(Gly-Gly-His)]$^+$.[18]

TABLE 2

Equilibrium constants of copper(II) complexes at 37 °C in I = 0.15 mol dm^{-3} ($K[NO_3]$) solutions

Equilibrium	log K ± s.d.
(1) Gly-Gly-His (pK_a values 2.43 ± 0.01, 6.52 ± 0.01, and 7.51 ± 0.01)	(s.d.t. 0.0022 cm^3)
$Cu^{2+} + L^- \rightleftharpoons [CuL]^+$	7.04 ± 0.02
$Cu^{2+} + HL \rightleftharpoons [Cu(HL)]^{2+}$	4.19 ± 0.03
$[CuL]^+ \rightleftharpoons [Cu(L - 2H)]^- + 2H^+$	−9.15 ± 0.02
$Cu^{2+} + 2L^- \rightleftharpoons [CuL(L - H)]^- + H^+$	8.70 ± 0.05
$[CuL(L - H)]^- \rightleftharpoons [Cu(L - H)_2]^{2-} + H^+$	−7.40 ± 0.06
(2) Gly-Gly-His-OMe (pK_a 6.24 ± 0.01 and 7.59 ± 0.01)	(s.d.t. 0.0017 cm^3)
$Cu^{2+} + L^- \rightleftharpoons [CuL]^+$	6.87 ± 0.04
$[CuL]^+ \rightleftharpoons [Cu(L - 2H)]^- + 2H^+$	−8.01 ± 0.04
$Cu^{2+} + 2L^- \rightleftharpoons [CuL(L - H)]^- + H^+$	9.21 ± 0.10
$[CuL(L - H)]^- \rightleftharpoons [Cu(L - H)_2]^{2-} + H^+$	−6.82 ± 0.05
(3) $PhCH_2OCO$-Gly-Gly-His-OMe (pK_a 6.23 ± 0.01)	(s.d.t. 0.0018 cm^3)
$Cu^{2+} + L^- \rightleftharpoons [CuL]^+$	3.49 ± 0.02
$[CuL]^+ \rightleftharpoons [Cu(L - 2H)]^- + 2H^+$	−11.78 ± 0.03
$[Cu(L - 2H)]^- \rightleftharpoons [Cu(L - 3H)]^{2-} + H^+$	−7.14 ± 0.03
(4) Gly-Gly-His-Gly-Gly (pK_a 3.16 ± 0.01, 6.15 ± 0.01, and 7.54 ± 0.01)	(s.d.t. 0.0017 cm^3)
$Cu^{2+} + L^- \rightleftharpoons [CuL]^+$	6.83 ± 0.05
$Cu^{2+} + HL \rightleftharpoons [Cu(HL)]^{2+}$	3.66 ± 0.05
$[CuL]^+ \rightleftharpoons [Cu(L - 2H)]^- + 2H^+$	−7.72 ± 0.01
$Cu^{2+} + 2L^- \rightleftharpoons [CuL(L - H)]^- + H^+$	9.38 ± 0.05
$[CuL(L - H)]^- \rightleftharpoons [Cu(L - H)_2]^{2-} + H^+$	−7.22 ± 0.04
(5) Gly-Gly-His-Gly-Gly-OEt (pK_a 6.13 ± 0.01 and 7.48 ± 0.01)	(s.d.t. 0.0010 cm^3)
$Cu^{2+} + L^- \rightleftharpoons [CuL]^+$	6.65 ± 0.03
$[CuL]^+ \rightleftharpoons [Cu(L - 2H)]^- + 2H^+$	−7.51 ± 0.01
(6) $PhCH_2OCO$-Gly-Gly-His-Gly-Gly-OEt (pK_a 6.20 ± 0.01)	(s.d.t. 0.0014 cm^3)
$Cu^{2+} + L^- \rightleftharpoons [CuL]^+$	3.60 ± 0.01
$[CuL]^+ \rightleftharpoons [Cu(L - 2H)]^- + 2H^+$	−11.48 ± 0.01
$[Cu(L - 2H)]^- \rightleftharpoons [Cu(L - 3H)]^{2-} + H^+$	−7.24 ± 0.01

* For details see Notices to Authors No. 7, *J.C.S. Dalton*, 1976, Index issue.

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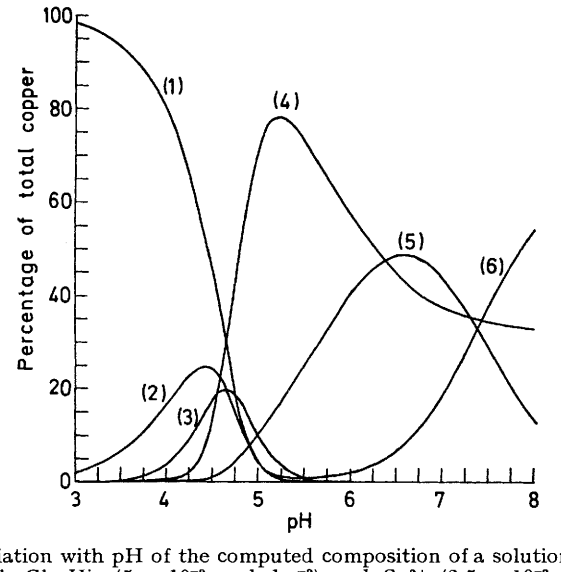
Our overall constant (log K −2.11 at 37 °C) for the reaction $Cu^{2+} + L^- \rightleftharpoons [Cu(L - 2H)]^- + 2H^+$ agrees with the published [8] value of −1.99 at 25 °C, and we also confirm that at pH ⩾ 6 the species $[Cu(L - 2H)]^-$ is the major complex in 1 : 1 solutions of Cu^{2+} and Gly-Gly-His. However, we doubt the reported [8] values of log K_1 9.22 at 25 °C for $[Cu(Gly\text{-}Gly\text{-}His)]^+$ and log K 3.65 at 25 °C for the reaction $Cu^{2+} + L^- \rightleftharpoons [Cu(L - H)] + H^+$ for two reasons: in the titrations from which these constants were derived the species $[Cu(L - H)]$ and $[CuL]^+$ 'never reached significant individual concentrations',[8] and the two constants give a pK_a for $[CuL]^+$ (5.57) which is not consistent with measured values. We suggest that the value of log K_1 is anomalously large and may be a composite of the constants for $[CuL]^+$ and $[Cu(HL)]^{2+}$.

In the presence of excess of Gly-Gly-His, appreciable amounts of the bis complexes $[CuL(L - H)]^-$ and $[Cu(L - H)_2]^{2-}$ were formed. The preference of Cu^{2+} for square-planar or distorted-octahedral complex formation suggests that in these bis complexes each ligand is only bi- or, perhaps, ter-dentate. It is likely that in $[CuL(L - H)]^-$ and $[Cu(L - H)_2]^{2-}$ each ligand is co-ordinated through its imidazole N′ and the adjoining (deprotonated) peptide nitrogen, while in $[CuL(L - H)]^-$ one of the terminal amino-groups is protonated. Similar structures would be expected for Cu^{2+} with Gly-His, except that the closer proximity of the charged amino-group to the metal ion would weaken the $[CuL(L - H)]^-$ complex of Gly-His. Values of log K for the reaction $Cu^{2+} + 2L^- \rightleftharpoons [CuL(L - H)]^- + H^+$ are



Variation with pH of the computed composition of a solution of Gly-Gly-His (5×10^{-3} mol dm^{-3}) and Cu^{2+} (2.5×10^{-3} mol dm^{-3}), as a percentage of the total copper present: (1), free Cu^{2+}; (2), $[Cu(HL)]^{2+}$; (3), $[CuL]^+$; (4), $[Cu(L - 2H)]^-$; (5), $[CuL(L - H)]^-$; (6), $[Cu(L - H)_2]^{2-}$

8.70, 7.68,[4] and 8.41 [4] for HL = Gly-Gly-His, Gly-His, and Gly-His-Gly, respectively. For Gly-Gly the value is only 4.47.[19] The Figure shows the computed pH–composition profile for a solution 0.0025 mol dm^{-3} in Cu^{II} and 0.005 mol dm^{-3} in Gly-Gly-His.

From Table 2 the types of copper complexes remain the same but there is a slight decrease in their stability constants when Gly-Gly-His-Gly-Gly is compared with Gly-Gly-His. A similar decrease is found in going from Gly-His to Gly-His-Gly.[4]

As was also observed with Gly-His,[4] titration curves for mixtures of Cu^{2+}, His, and Gly-Gly-His or Gly-Gly-His-Gly-Gly showed ternary complex formation to be unimportant when the reactants were present in approximately equal concentrations. However, Kruck and Sarkar,[20] using higher ligand–metal ratios, obtained refined stability constants for a set of five ternary complexes $[CuH_n(HisO)L]$ (n = 2, 1, 0, −1, or −2; HisO = histidine anion, L = Gly-Gly-His) which have similar values to some bis(histidinato) and bis(glycylglycylhistidine) complexes. Thus log K for [Cu(HisO)L] (17.56 at 25 °C) [20] is similar to log β_2

TABLE 3

Equilibrium constants of zinc(II) complexes at 37 °C in I = 0.15 mol dm^{-3} ($K[NO_3]$) solutions

Equilibrium	log K ± s.d.
(1) Gly-Gly-His	(s.d.t. 0.0020 cm³)
$Zn^{2+} + L^- \rightleftharpoons [ZnL]^+$	3.31 ± 0.06
$Zn^{2+} + HL \rightleftharpoons [Zn(HL)]^{2+}$	2.57 ± 0.04
$2[ZnL]^+ \rightleftharpoons [Zn_2L_2]^{2+}$	3.15 ± 0.09
$[Zn_2L_2]^{2+} \rightleftharpoons [Zn_2L(L - H)]^+ + H^+$	−6.46 ± 0.04
$[Zn_2L(L - H)]^+ \rightleftharpoons [Zn_2(L - H)_2] + H^+$	−7.80 ± 0.03
(2) Gly-Gly-His-OMe	(s.d.t. 0.0021 cm³)
$Zn^{2+} + L^- \rightleftharpoons [ZnL]^+$	3.00 ± 0.03
$[ZnL]^+ \rightleftharpoons [Zn(L - H)] + H^+$	−7.12 ± 0.02
(3) $PhCH_2OCO$-Gly-Gly-His-OMe	(s.d.t. 0.0025 cm³)
$Zn^{2+} + L^- \rightleftharpoons [ZnL]^+$	1.81 ± 0.05
(4) Gly-Gly-His-Gly-Gly	(s.d.t. 0.0012 cm³)
$Zn^{2+} + L^- \rightleftharpoons [ZnL]^+$	2.91 ± 0.04
$Zn^{2+} + HL \rightleftharpoons [Zn(HL)]^{2+}$	1.92 ± 0.03
$[ZnL]^+ \rightleftharpoons [Zn(L - H)] + H^+$	−7.14 ± 0.03
$2[ZnL]^+ \rightleftharpoons [Zn_2L_2]^{2+}$	3.24 ± 0.06
$[Zn_2L_2]^{2+} \rightleftharpoons [Zn_2L(L - H)]^+ + H^+$	−6.82 ± 0.02
(5) Gly-Gly-His-Gly-Gly-OEt	(s.d.t 0.0013 cm³)
$Zn^{2+} + L^- \rightleftharpoons [ZnL]^+$	3.04 ± 0.02
$[ZnL]^+ \rightleftharpoons [Zn(L - H)] + H^+$	−7.08 ± 0.01

(17.41 at 37 °C) [21] for $[Cu(HisO)_2]$ and log K for the reaction $Cu^{2+} + 2L^- \rightleftharpoons [Cu(L - H)_2]^{2-} + 2H^+$ (1.30 at 37 °C, this work) compares with 1.7 (at 25 °C) for $[CuH_{-2}(HisO)L]$.[20] On the other hand, no constant has been found for the complex $[CuL_2]$, corresponding to [Cu(HisO)L] and $[Cu(HisO)_2]$, although a value of log β_2 ~17 would be expected.

The stability constants listed in Table 3 for complexes of Zn^{2+} with Gly-Gly-His were obtained by computer analysis of titration data. The complexes appear to be of the same types as for zinc with Gly-His [5] and corresponding constants are similar in value. Thus log K_1 = 3.65 for $[Zn(Gly\text{-}His)]^+$ (ref. 5) and 3.31 for $[Zn(Gly\text{-}Gly\text{-}His)]^+$ {compare 3.24 for $[Zn(Gly\text{-}Gly)]^+$ and 3.00 for $[Zn(Gly\text{-}Gly\text{-}Gly)]^+$} and log K = 3.30 and 3.15,[5] respectively, for the dimerisation reaction $2[ZnL]^+ \rightleftharpoons [Zn_2L_2]^{2+}$. Both peptides form binuclear complexes with zinc. The dimers $[Zn_2L_2]^{2+}$ lose two protons, probably by ionisation of peptide hydrogens, with pK_a values [5] of 6.19 and 7.89 for Gly-His and 6.46 and 7.80 for Gly-Gly-His.

The slight decrease in log K_1 in going from $[Zn(Gly\text{-}Gly\text{-}His)]^+$ to its ester or to $[Zn(Gly\text{-}Gly\text{-}His\text{-}Gly\text{-}Gly)]^+$ shows that the carboxyl group is not important as a binding site for the zinc. Conversely, the big decrease in log K_1 when the methyl ester of Gly-Gly-His is benzyloxycarbonylated points to the involvement of the primary amino-group in metal binding.

Quantitative interpretation of pH-titration curves for

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solutions approximately equimolar in Zn^{2+}, His, and Gly-Gly-His required the postulation of two mixed species, $Zn^{2+} + H^+ + HisO^- + L^- \rightleftharpoons [ZnH(HisO)L]^+$ (log K 15.91) and $Zn^{2+} + HisO^- + L^- \rightleftharpoons [Zn(HisO)L]$ (log K 9.37). When the peptide was Gly-Gly-His-Gly-Gly the only mixed-ligand species detected was [Zn(HisO)L] (log K 9.05). These values of the logarithm of the stability constants of [Zn(HisO)L] are not very different from the value calculated from the mean of log β_2 for [Zn(Gly-His)$_2$] and [Zn(His)$_2$], $\frac{1}{2}(6.89 + 11.49) = 9.19$,[5] by adding a statistical factor of log 2 = 0.30.

TABLE 4

Some conditional constants of copper and zinc complexes, valid at pH 7.4 and 37 °C

		log K'
(1) Albumin [22]	[Cu(alb)]	12.04
	[Cu(alb)$_2$]	16.90
	[Cu(GlyO)(alb)]	15.23
	[Cu(HisO)(alb)]	16.13
	[Zn(alb)]	7.60
	[Zn$_2$(alb)]	13.51
(2) Gly-Gly-His	[Cu(L − 2H)]$^-$	12.3
	[Cu(L − H)$_2$]$^{2-}$ + [CuL(L − H)]$^-$	15.7
	[ZnL]$^+$	3.0
	[Zn$_2$L$_2$]$^{2+}$	9.1
(3) Gly-Gly-His-OMe	[Cu(L − 2H)]$^-$	13.3
	[Cu(L − H)$_2$]$^{2-}$ + [CuL(L − H)]$^-$	16.6
	[ZnL]$^+$ + [Zn(L − H)]	3.1
(4) Gly-Gly-His-Gly-Gly	[Cu(L − 2H)]$^-$	13.6
	[Cu(L − H)$_2$]$^{2-}$ +	16.5
	[CuL(L − H)]$^-$	16.5
	[ZnL]$^+$ +	3.0
	[Zn(L − H)]	3.0
	[Zn$_2$L$_2$]$^{2+}$	8.3

The effectiveness of Gly-Gly-His and Gly-Gly-His-Gly-Gly as metal-binding models for albumin at pH 7.4 and 37 °C was examined using recent values [22] for equilibria in mixtures of Zn^{2+} or Cu^{2+} with bovine serum albumin (alb). These constants were obtained from competitive dialysis of pairs of pH-buffered solutions containing varying concentrations of metal ion and amino-acid in one compartment and metal ion, amino-acid, and albumin in the other. After dialysis for 4—8 h, the copper or zinc concentrations were determined in each half-cell and Fick's law was used to extrapolate to equilibrium conditions.[22] Pairs of solutions approximating to these equilibrium compositions were then prepared and dialysed, the procedure being repeated with new solutions until there was no further change on dialysis. We consider this method to be more reliable than an earlier one [23] in which a solution containing metal ions and albumin was left in prolonged contact at 6 °C with a buffered His solution (not initially containing copper or zinc) until equilibrium was assumed to be reached. This assumption was also made when Gly-Gly-His was used instead of His to compare the binding of Cu^{2+} by Gly-Gly-His and albumin.[8] A conditional constant of log K' = 15.9 at pH 7.5 for [Cu(Gly-Gly-His)] was based on a value of log K' = 16.2 for [Cu(alb)].[23]

'Conditional' constants, as defined by Ringbom,[24] are valid for specified conditions and have the advantages of avoiding uncertainties in the extent of protonation of ligands and complexes and of permitting direct comparison, at a given pH, of metal-binding abilities of different ligands. Thus the species [Cu(alb)] comprises all 1 : 1 copper–albumin complexes, irrespective of their proton status. Similarly, instead of free-ligand concentration, the total concentration of uncomplexed albumin is used in equilibria calculations. The constants listed in Table 4 are valid for pH 7.4 and 37 °C. Table 4 also lists conditional constants for the corresponding metal–peptide complexes, derived from the constants in Tables 2 and 3.

The constants for copper(II)– and zinc(II)–albumin complexes and the average plasma concentration of albumin (4.7 g per 100 cm^3 = 7 × 10^{-4} mol dm^{-3}) [25] were inserted into the model used earlier [26] to compute the distribution of exchangeable Cu^{2+} and Zn^{2+} among 22 amino-acids in

TABLE 5

Computed extent of binding of Cu^{2+} and Zn^{2+} by albumin, Gly-Gly-His, or Gly-Gly-His-Gly-Gly at 37 °C and pH 7.4, in competition with 22 amino-acids. Constants and total concentrations were from refs. 22 and 26 and the present work. The concentration of each complex is expressed as a percentage of the total copper(II) or zinc(II) present

		Percentage of $[Cu]_T$		Percentage of $[Zn]_T$
(1) Albumin	[Cu(alb)]	8.0	[Zn(alb)]	97.9
	[Cu(alb)$_2$]	91.3	[Zn$_2$(alb)]	0.8
	[Cu(HisO)(alb)]	0.4		
	Total	99.7		98.7
Free Cu 3.9 × 10^{-16} mol dm^{-3}		Free Zn 5.0 × 10^{-9} mol dm^{-3}		
(2) Gly-Gly-His	[Cu(L − 2H)]$^-$	40.1	[Zn(HL)]$^{2+}$	0.8
	[CuL(L − H)]$^-$	29.5	[ZnL]$^+$	3.1
	[Cu(L − H)$_2$]$^{2-}$	30.2	[Zn(HisO)(HL)]$^+$	0.6
			[Zn(HisO)L]	4.5
	Total	99.8		9.0
Free Cu 3.1 × 10^{-16} mol dm^{-3}		Free Zn 2.4 × 10^{-6} mol dm^{-3}		
(3) Gly-Gly-His-Gly-Gly	[Cu(L − 2H)]$^-$	65.5	[Zn(HL)]$^{2+}$	0.2
	[CuL(L − H)]$^-$	13.7	[ZnL]$^+$	1.3
	[Cu(L − H)$_2$]$^{2-}$	20.8	[Zn(L − H)]	2.3
			[Zn(HisO)L]	2.3
	Total	99.99		6.1
Free Cu 3.1 × 10^{-17} mol dm^{-3}		Free Zn 2.5 × 10^{-6} mol dm^{-3}		

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[26] D. D. Perrin and R. P. Agarwal, 'Metal Ions in Biological Systems,' ed. H. Sigel, Marcel Dekker, New York, 1973, vol. 2, ch. 4.

blood plasma. The effect of replacing albumin by Gly-Gly-His or Gly-Gly-His-Gly-Gly was similarly computed. Percentages of the Cu^{2+} and Zn^{2+} bound to the albumin and the peptides, respectively, are given in Table 5. The peptide Gly-Gly-His approximates closely to albumin in total copper-binding ability, thus providing supporting evidence for the involvement of the Asp-Ala-His portion of albumin and confirming equilibrium dialysis results that Gly-Gly-His is able to compete with albumin for Cu^{2+}.[8] Although replacement of albumin by Gly-Gly-His produced little effect on the computed free copper(II) concentration in the plasma model, much greater differences were observed in the free zinc(II) concentration so that this peptide is a poor model for albumin in its zinc-bonding ability. That Gly-Gly-His-Gly-Gly forms more stable copper(II) complexes than albumin may be due to the bulkiness of the albumin molecule and to greater crowding when Cu^{2+} is bound to the Asp-Ala-His-Lys-Ser moiety.

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