The Computed Distribution of Copper(II) and Zinc(II) Ions Among Seventeen Amino Acids Present in Human Blood Plasma

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The equilibrium distribution of copper(II) and zinc(II) ions among a mixture of 17 amino acids has been computed from stability-constant and blood-plasma-composition data. At pH 7.4, 98% of the copper(II) in the simulated plasma solution is co-ordinated to histidine and cystine, predominantly as the mixed-ligand complexes $[\text{Cu} \cdot \text{His} \cdot \text{Cystine}]^-$ and $[\text{Cu} \cdot \text{H} \cdot \text{His} \cdot \text{Cystine}]$. Approximately half of the zinc(II) is co-ordinated to cysteine and histidine, but appreciable complex-formation occurs with most of the other amino acids. Stability constants are given for copper(II) and zinc(II) amino acid complexes, including some mixed-ligand species, at 37°C and $I = 0.15\text{M}$.

Although metal ions and complexing agents occur ubiquitously in biological tissues and fluids, few studies have been made of the distribution of the metal ions among the competing ligands in such systems. Until recently, all comments were qualitative. Thus Orgel (1958) made an 'informed guess' about the likely co-ordination partners for particular metal ions and the amino acid residues in proteins. Similarly, Williams (1959) deduced 'the most likely co-ordination partner for a given cation at pH 7.3' in biological systems. The first quantitative study of metal binding in a multi-ligand system under physiological conditions appears to have been that of Neumann & Sass-Kortsak (1967) who, by ultracentrifugation and the use of ^{64}Cu , showed that in the competition between albumin and amino acids for copper(II) in blood serum, copper(II) is co-ordinated preferentially to histidine in both simple and mixed complexes.

An alternative approach is possible by using computer programs designed to solve multiple simultaneous equations (Perrin, 1965; Perrin & Sayce, 1967; Ingri, Kakolowicz, Sillén & Warnqvist, 1967, 1968). Given the total concentrations of the different metal ions and complexing agents, the pH of the solution and the relevant equilibrium constants (pK_a and stability-constant values), these programs compute the distribution of metal ions among the ligands in such a way that all equilibria are satisfied. Such programs are well suited to the quantitative study of complex equilibria in bio-

logical solutions. Some possible applications include the interpretation of the metal-ion activation of enzymes *in vivo*, the masking and removal of toxic cations, and kinetic studies of ion transport.

We now report a calculation of the distribution of copper(II) and zinc(II) ions among seventeen amino acids present in human plasma, where it is known that amino acids can compete effectively with albumin for the binding of exchangeable copper(II) (Neumann & Sass-Kortsak, 1967).

As an essential preliminary, stability constants of the metal complexes were determined at 37°C and an ionic strength of 0.15 because few of the previously published constants (Sillén & Martell, 1964) had been obtained under these conditions. Potassium nitrate, rather than sodium chloride, was used as supporting electrolyte because, in a subsequent study, we intend to examine the behaviour of chloride ion as a complexing agent.

EXPERIMENTAL

Materials. L-Ornithine hydrochloride, L-leucine, L-isoleucine, L-glutamic acid, L-arginine and L-tryptophan were from Sigma Chemical Co., St Louis, Mo., U.S.A. L-Cystine, L-cysteine and L-histidine (Puriss grade) were from Fluka A.G. Chemische Fabrik, Buchs, Switzerland. L-Methionine (A grade) was from Calbiochem, Los Angeles, Calif., U.S.A. L-Glutamine (laboratory reagent) and glycine (AnalaR) were from BDH Chemicals Ltd., Poole, Dorset, U.K. Cystine, recrystallized from 1M-HCl solution by the addition of 1M-KOH, was dried at 100°C for 2h and then stored *in vacuo* at room temperature. Histidine, glycine, glutamic acid and leucine were recrystallized from aqueous ethanol and dried at 110°C.

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The other amino acids were dried before use. The purity of cysteine, checked by an iodometric method (Perrin & Sayce, 1968), was greater than 99%. Solutions of this amino acid were freshly prepared under nitrogen when required. Boiled-out glass-distilled water was used to prepare all solutions.

KNO₃ (AnalaR) was recrystallized from water and dried overnight at 120°C. KOH solutions were prepared and standardized as described previously (Childs & Perrin, 1969). Cu(NO₃)₂ and Zn(NO₃)₂ solutions were assayed complexometrically (Schwarzenbach & Flaschka, 1969).

Methods. The pH titration apparatus and techniques used to determine 'practical' pK_a and stability-constant values have been discussed elsewhere (Perrin & Sayce, 1966; Childs & Perrin, 1969). Constants were evaluated with the aid of the computer program SCOGS (Sayce, 1968) on an IBM 360/50 computer. The quantity *F* (set equal to 0.80) that is needed in this program to determine free hydrogen ion concentrations from pH-meter readings is essentially the activity coefficient of the hydrogen ion, but includes any residual liquid-junction potential and small systematic errors (Childs & Perrin, 1969). The program COMICS (Perrin & Sayce, 1967) was used to calculate the equilibrium concentrations of the species present in solutions containing mixtures of metal ions and amino acids.

RESULTS AND DISCUSSION

'Practical' pK_a values for the amino acids studied are given in Table 1. The numerically greatest pK_a value for ornithine is assigned to the terminal amino group. Except for the experimentally determined constants for arginine, isoleucine, leucine and ornithine, the pK_a values near to 2 have been determined from published thermodynamic values by taking activity coefficients of H₂L⁺ and HL[±] as 0.75 and 0.84 (Childs & Perrin, 1969); their effects on the stability constant calculations are only marginal.

Although the complexes ML, ML₂ and ML₃ usually account for most of the metal-binding by amino acids, previous work (Perrin & Sharma, 1967; Perrin & Sayce, 1968; Childs & Perrin, 1969) has shown that the formation of protonated, hydrolysed and polynuclear complexes must also be considered. This has been done in obtaining the stability constants listed in Table 2. Constants, and the reactions to which they apply, are defined as follows:

Table 1. 'Practical' pK_a values of L-amino acids at 37°C, *I* 0.15M (potassium nitrate)

Values in parentheses are estimates.		
Amino acid	pK _a values	Reference
Alanine	9.50, 2.38	Childs & Perrin (1969)
Arginine	8.82 ± 0.005, 1.82	Present work
Cysteine	10.23 ± 0.01, 8.07 ± 0.01, (1.90)	Present work
Cystine	8.69, 7.95, (1.7)	Present work
Glutamic acid	9.39, 4.15, (2.13)	Present work
Glutamine	8.83, 2.15	Present work
Glycine	9.38, 2.38	Childs & Perrin (1969)
Histidine	8.92, 5.96, (2.24)	Perrin & Sharma (1967)
Isoleucine	9.36 ± 0.01, 2.36	Present work
Leucine	9.36 ± 0.01, 2.36	Present work
Methionine	8.91, 2.255	Present work
Ornithine	10.22 ± 0.01, 8.55 ± 0.01, 1.92	Present work
Proline	10.34, 2.005	Childs & Perrin (1969)
Serine	8.84, 2.18	Perrin <i>et al.</i> (1967)
Threonine	8.71, 2.20	Sharma (1967)
Tryptophan	9.085, 2.46	Present work
Valine	9.32, 2.34	Childs & Perrin (1969)

Table 2. Stability constants of copper and zinc complexes with L-amino acids at 37°C, I 0.15 M (potassium nitrate)

Amino acid	Metal	Constants				Ref.
		$\log K_1$	$\log \beta_2$	$\log K_1'$	Others	
Alanine	Cu	8.01	14.64	1.07	$\log K_{aML} = -8.53$ $\log \beta_{M_2(OH)_{1,2}} = 3.17 \pm 0.04$ $\log K_{aML} = -4.25 \pm 0.11$ $\log \beta_{24}' = 38.4 \pm 0.07$ $\log \beta_2' = 14.1 \pm 0.03$ $\log \beta_2'' = 9.4 \pm 0.03$ $\log \beta_{22} = 28.07 \pm 0.01$	Childs & Perrin (1969)
	Zn	4.57	8.56			Childs & Perrin (1969)
	Cu	7.38 $\pm$ 0.01	13.66 $\pm$ 0.01			Present work
	Zn	4.03 $\pm$ 0.02	7.56 $\pm$ 0.06			Present work
	Zn		17.98 $\pm$ 0.01			Present work
Cysteine						
Cystine	Cu			7.51 $\pm$ 0.03		Present work
Glutamic acid	Cu	8.74	14.91	3.40		Present work
	Cu	4.76	8.54			Present work
	Zn	7.24	13.40			Present work
Glutamine	Cu	4.27	7.94			Present work
Glycine	Cu	8.02	14.67	0.73		Childs & Perrin (1969)
	Zn	4.90	9.01	0.12	11.31	Childs & Perrin (1969)
	Cu	9.80 $\pm$ 0.01	17.50 $\pm$ 0.02	5.00 $\pm$ 0.02	$\log K_{aML} = -8.89$ $\log \beta_2' = 14.23 \pm 0.02$ $\log K_{aML} = -7.34 \pm 0.07$ $\log \beta_{M_2(OH)_{1,2}} = 7.38$	Perrin & Sharma (1967)*
Histidine						
Isoleucine	Zn	6.32 $\pm$ 0.01	11.68 $\pm$ 0.03	2.20 $\pm$ 0.08		Perrin & Sharma (1967)*
	Cu	7.95 $\pm$ 0.01	14.68 $\pm$ 0.01		$\log K_{aML} = -7.63 \pm 0.06$	Present work
	Zn	4.40 $\pm$ 0.04	8.08 $\pm$ 0.06		$\log K_{aML} = -8.02 \pm 0.09$ $\log \beta_2' = 5.89 \pm 0.36$	Present work
Leucine	Cu	8.04 $\pm$ 0.04	14.69 $\pm$ 0.05	2.13 $\pm$ 0.14		Present work
	Zn	4.51 $\pm$ 0.02	8.56 $\pm$ 0.01		$\log \beta_2' = 10.07 \pm 0.12$ $\log K_{aML} = -8.76 \pm 0.10$ $\log \beta_2' = 5.81 \pm 0.16$	Present work
Methionine	Cu	7.67	14.08			Present work
	Zn	4.22	6.93			Present work
	Cu	9.78 $\pm$ 0.28	14.77 $\pm$ 0.06	7.20 $\pm$ 0.03	$\log \beta_2' = 14.51 \pm 0.02$ $\log \beta_2'' = 13.20 \pm 0.01$ $\log K_{aML} = -8.89 \pm 0.16$	Present work
Ornithine						
	Zn	5.96 $\pm$ 0.05		3.86 $\pm$ 0.03	$\log \beta_2' = 9.09 \pm 0.03$ $\log \beta_2'' = 7.18 \pm 0.03$ $\log K_{aML} = -8.55 \pm 0.03$	Present work
Proline	Cu	8.67	16.00	0.27		Childs & Perrin (1969)
	Zn	5.13	9.69		11.16	Childs & Perrin (1969)
Serine	Cu	7.56	14.01			Sharma (1967)
	Zn	4.47	8.31			Sharma (1967)
	Cu	7.55	14.01		10.56	Sharma (1967)
Threonine	Zn	4.43	8.14		10.09	Sharma (1967)
	Cu	8.05	15.32			Present work
Tryptophan	Zn	4.50	8.76		11.61	Present work
	Cu	7.90	14.55			Childs & Perrin (1969)
	Zn	4.44	8.24		10.62	Childs & Perrin (1969)
Valine						
					$\log K_{aML} = -8.62 \pm 0.14$	

* Recalculated for F = 0.80.

The bis-ornithine complexes with zinc are protonated on one or both of the δ -amino nitrogens: no constant could be obtained for the corresponding bis complex in which neither terminal amino group was protonated.

Mixed-ligand complex-formation occurs widely in systems containing metal ions and two or more different ligands (Martin & Paris, 1963, 1964; Perrin, Sayce & Sharma, 1967; Perrin & Sharma, 1968). For related ligands the stability constant of a mixed complex, MAB, is usually comparable with, or slightly greater than, the mean of the constants for the species MA_2 and MB_2 . From preliminary calculations, with constants obtained in single-metal, single-ligand systems, together with data on plasma composition, mixed-complex formation appeared likely to be important for copper-cystine-histidine, zinc-cystine-histidine, zinc-cystine-histidine, zinc-glutamic acid-histidine and zinc-glycine-histidine. The relevant constants, obtained from pH-titration data on appropriate solutions are summarized in Table 3. The constants are defined as follows:

$$\begin{aligned} K_{111} &= [MAB]/[M][A][B] \\ K_{111}' &= [MHAB]/[M][A][B](H^+) \\ K_{111}'' &= [MH_2AB]/[M][A][B](H^+)^2 \\ \beta_{112}' &= [MHAB_2]/[M][A][B]^2(H^+) \\ \beta_{212}' &= [M_2HAB_2]/[M]^2[A][B]^2(H^+) \end{aligned}$$

We have so far been unable to determine the stability constants of zinc-cystine complexes, because of their insolubility, and copper(II)-cystine complexes, because of their reactivity. Also, in the

zinc-cysteine system the second stepwise formation constant is greater than the first, so that $\log \beta_2$, but not $\log K_1$, has been obtained experimentally (Perrin & Sayce, 1968). However, titrations of zinc, cystine and histidine in molar ratios of 1:1:1 and 1:1:2 [$Zn(II) = 0.18 \text{ mM}$] afforded constants for mixed-ligand zinc-cystine-histidine complexes. Although cysteine ordinarily reduces copper(II) to copper(I), with the formation of cystine (Greenstein & Winitz, 1961), it is not at present known whether, in plasma, the presence of ligands which preferentially stabilize copper(II) can lower the oxidation-reduction potential of copper(II)/copper(I) sufficiently for reaction with cysteine to be incomplete or, possibly, prevented. Studies to be reported elsewhere showed that in ammoniacal solution a mixed-valence compound of copper(II) and copper(I) is formed in the presence of limited concentrations of cysteine.

Table 4 lists average values for 'direct reacting' copper(II), zinc(II) and amino acid concentrations in human blood plasma. Most methods of amino acid analysis report only the total cystine plus cysteine. However, Brigham, Stein & Moore (1960) used prior reaction with iodoacetic acid to prevent conversion of cysteine into cystine, and their values have been taken. For purposes of calculation, the copper bound in caeruloplasmin was assumed to be non-exchangeable (Cartwright & Wintrobe, 1964). The pH of the solution was taken as 7.4. Possible hydrolysis of copper(II) and zinc(II) ions was allowed for by including values of the appropriate

Table 3. *Stability constants of mixed-ligand complexes at 37°C, I 0.15 M*

Amino acid abbreviations refer to the anionic form, e.g. Gly = $NH_2CH_2CO_2^-$. Cystine is not abbreviated and, in the formulae below, denotes $[-O_2CCH(NH_2)CH_2S-]_2$.

Complex	Constant
$[Cu \cdot \text{Cystine} \cdot \text{His}]^-$	$\log K_{111} = 18.51 \pm 0.05$
$[Cu \cdot H \cdot \text{Cystine} \cdot \text{His}]$	$\log K_{111}' = 25.80 \pm 0.01$
$[Cu \cdot H_2 \cdot \text{Cystine} \cdot \text{His}]^+$	$\log K_{111}'' = 30.69 \pm 0.03$
$[Cu_2 \cdot H \cdot \text{Cystine} \cdot (\text{His})_2]^+$	$\log \beta_{212}' = 39.15 \pm 0.15$
$[Zn \cdot \text{Cystine} \cdot \text{His}]^-$	$\log K_{111} = 11.62 \pm 0.02$
$[Zn \cdot H \cdot \text{Cystine} \cdot \text{His}]$	$\log K_{111}' = 19.55 \pm 0.01$
$[Zn \cdot H_2 \cdot \text{Cystine} \cdot \text{His}]^+$	$\log K_{111}'' = 26.35 \pm 0.02$
$[Zn \cdot \text{Cys} \cdot \text{His}]^-$	$\log K_{111} = 15.23 \pm 0.02$
$[Zn \cdot H \cdot \text{Cys} \cdot \text{His}]$	$\log K_{111}' = 21.60 \pm 0.02$
$[Zn \cdot H \cdot \text{Cys} \cdot (\text{His})_2]$	$\log \beta_{112}' = 26.5 \pm 0.3$
$[Zn \cdot \text{Glu} \cdot \text{His}]^-$	$\log K_{111} = 10.75 \pm 0.04$
$[Zn \cdot H \cdot \text{Gly} \cdot \text{His}]^+$	$\log K_{111}' = 17.72 \pm 0.04$
$[Zn \cdot H \cdot (\text{Gly})_2 \cdot \text{His}]$	$\log \beta_{121}' = 23.10 \pm 0.04$
$[Zn \cdot H \cdot \text{Gly} \cdot (\text{His})_2]$	$\log \beta_{112}' = 22.79 \pm 0.06$

Table 4. *Average concentrations of free amino acids and total metal ions in human blood plasma*

	Concn. (mM)	Reference
Alanine	0.383	Stein & Moore (1954)
Arginine	0.086	Stein & Moore (1954)
Cysteine	0.033	Brigham <i>et al.</i> (1960)
Cystine	0.042	Brigham <i>et al.</i> (1960)
Glutamic acid	0.048	Stein & Moore (1954)
Glutamine	0.568	Archibald (1944)
Glycine	0.205	Stein & Moore (1954)
Histidine	0.074	Stein & Moore (1954), Hill & Mills (1961)
Isoleucine	0.068	Stein & Moore (1954)
Leucine	0.129	Stein & Moore (1954)
Methionine	0.026	Stein & Moore (1954)
Ornithine	0.055	Stein & Moore (1954)
Proline	0.205	Stein & Moore (1954)
Serine	0.107	Stein & Moore (1954)
Threonine	0.117	Stein & Moore (1954)
Tryptophan	0.054	Heir & Bergeim (1946)
Valine	0.246	Stein & Moore (1954)
Copper(II)	0.0011	Cartwright & Wintrobe (1964)
Zinc(II)	0.046	Vallee & Gibson (1948)

Table 5. *Computed distribution of Cu(II) and Zn(II) ions among 17 amino acids*

The standard distribution (a) was computed by using the constants given in Tables 1, 2, 3 and the concentrations shown in Table 4. The other distributions were obtained when one of the concentrations was increased by its (published) standard deviation. For (b) His = 94 μ M; for (c) Zn(II) = 70 μ M; and for (d) Cu(II) = 2.2 μ M. The concentration of each complex is expressed as a percentage of the total copper(II) or zinc(II) present, and only those complexes containing at least 0.2% of the total metal ion concentration are shown.

Complex	Distribution			
	(a) (%)	(b) (%)	(c) (%)	(d) (%)
[Cu·Cystine·His] ⁻	48	46	49	48
[Cu·H·Cystine·His]	37	35	38	37
[Cu(His) ₂]	13	18	10	13
[Cu(Gln) ₂]	0.3	0.2	0.5	0.3
[Cu·OH·His]	0.3	0.3	0.3	0.3
[Cu·H·(His) ₂] ⁺	0.2	0.3	0.2	0.2
[Cu·His] ⁺	0.2	0.2	0.3	0.2
[Zn·His·Cys] ⁻	24	27	17	24
[Zn·His] ⁺	21	22	23	21
[Zn(Cys) ₂] ²⁻	16	14	11	16
Zn ²⁺	8.6	6.5	13.4	8.8
[Zn·H·His·Cystine]	5.8	6.1	6.1	5.7
[Zn(His) ₂]	5.7	8.1	4.5	5.5
[Zn·Gln] ⁺	3.3	2.5	5.1	3.3
[Zn·H·Cys·His]	2.2	2.5	1.6	2.2
[Zn·His·Cystine]	1.7	1.8	1.8	1.7
[Zn·H ₂ ·His·Cystine]	1.5	1.5	1.5	1.4
[Zn·Gly] ⁺	1.4	1.1	2.2	1.5
[Zn·H·(Cys) ₂] ⁻	1.3	1.1	0.9	1.3
[Zn·Thr] ⁺	1.3	1.0	2.0	1.3
[Zn·Ser] ⁺	1.0	0.7	1.5	1.0
[Zn·Ala] ⁺	1.0	0.7	1.5	1.0
[Zn·Val] ⁺	0.7	0.5	1.1	0.7
[Zn·H·Gly·His] ⁺	0.5	0.5	0.5	0.5
[Zn·Leu] ⁺	0.4	0.3	0.6	0.4
[Zn(Gln) ₂]	0.3	0.2	0.5	0.3
[Zn·Trp] ⁺	0.3	0.2	0.5	0.3
[Zn·Pro] ⁺	0.3	0.2	0.4	0.3
[Zn·Arg] ⁺	0.3	0.2	0.4	0.3
[Zn·Glu·His] ⁻	0.3	0.3	0.3	0.3
[ZnOH] ⁺	0.3	0.2	0.4	0.3
[Zn·Glu]	0.2	0.2	0.4	0.2
[Zn·H·Orn]	0.2	0.2	0.4	0.2
[Zn·H·His·(Gly) ₂]	0.2	0.2	0.3	0.2
[Zn·H·(His) ₂ ·Cys] ⁻	0.2	0.3	0.1	0.2

constants obtained by interpolation from the literature (Perrin, 1960, 1962).

Equilibrium concentrations of metal complexes, free metal ions and free amino acid anions have been computed, by using the constants in Tables 1, 2 and 3 (a total of 159 in all), for a solution at pH 7.4 with the composition given in Table 4. Results are listed in Tables 5 and 6. Complexes have been omitted

Table 6. *Equilibrium concentrations of free amino acids and free metal ions*

Values are the computed equilibrium concentrations of free amino acid anion and free metal ions present at pH 7.4 in a solution with the composition shown in Table 4.

Anion	Concn. (μ M)	Anion	Concn. (μ M)
Ala	3.0	Arg	3.1
Cys	0.0014	Cystine	0.41
Glu	0.48	Gln	20
Gly	2.1	His	1.2
Ile	0.72	Leu	1.4
Met	0.79	Orn	0.0052
Pro	0.24	Ser	3.7
Thr	5.4	Trp	1.1
Val	2.9		
Cu ²⁺	0.34*	Zn ²⁺	4.0

* pm.

from Table 5 if they contain less than 0.2% of the relevant total metal ion.

To assess the effects of variation in composition, similar calculations were carried out after changing the concentrations of histidine (to 94 μ M), copper (to 2.2 μ M), or zinc (to 70 μ M) by amounts equal to their reported standard deviations, and these results are also given in Table 5. The above alterations did not significantly change the metal ion distribution patterns, but with the increased zinc concentration there were decreases in the percentage of zinc present as Zn(Cys)₂²⁻ and Zn·His·Cys⁻, as well as in Cu(His)₂, reflecting the relatively low total histidine and cysteine concentrations. Similar calculations showed that, at the normal zinc and copper concentrations, small variations in cysteine or cystine concentrations had only marginal effects.

Although the present study considers the formation of 52 different copper complexes with the 17 amino acids listed, three complexes account for more than 98% of the bound copper(II), with histidine and cystine being the most successful of the competing ligands. The mixed-ligand complex Cu·His·Cystine⁻ and its monoprotinated derivative are the major copper species present. Because so few species are present in appreciable amounts, varying the values of the stability constants by up to several times their estimated standard deviations had a negligible effect on the metal ion distribution. Zinc(II) is distributed more evenly among the amino acids, so that 27 of the 68 possible complexes contain at least 0.2% of the total zinc present. Histidine and cysteine are the preferred amino acids, but the possibility must not be overlooked that zinc-cystine complex formation may also be important.

The success of histidine, cystine and cysteine in competing for the available metal ions is undoubtedly due to their ability to act as tridentate

ligands, whereas most α -amino acids can form only bidentate chelates. Thus, the crystal structure of a mixed-ligand complex, Cu·His·Thr (Freeman *et al.* 1969) shows the copper to be co-ordinated to the histidine through an imidazole nitrogen as well as the amino nitrogen and a carboxyl oxygen. The strongest bonding appears to be through the nitrogen atoms. Similarly, in copper(II)-cystine complexes it is believed that, as well as the usual metal-amino acid bonding, interaction occurs between the metal ion and the -S-S- linkage (Hawkins & Perrin, 1963). In Cu·His·Cystine⁻ the copper is probably co-ordinated to the histidine in the same way as for Cu·His·Thr, and the bonding to cystine is probably the same as in copper(II)-cystine complexes. We suggest that in the structure of Cu·His·Cystine⁻ two nitrogens of the histidine and a nitrogen and carboxyl oxygen of cystine lie in a plane around the copper ion, the fifth and sixth co-ordination sites of which are occupied, less strongly, by the histidine carboxyl group and the -S-S- portion of cystine. N.m.r. evidence has been presented to show that the cysteine anion is a tridentate ligand in which sulphur, nitrogen and oxygen donor atoms co-ordinate to the metal ion (McCormick, Sigel & Wright, 1969).

Similarly, whereas protonation of most α -amino acids lets them function only as monodentate ligands, monoprotection of histidine, cystine or cysteine leaves them able to act as bidentate chelating agents, with corresponding gain in stability of their complexes. It is likely, for example, that in the species Cu·H·His·Cystine the proton is on the amino group of the cystine molecule at the end remote from the copper.

Copper complexes are much more stable than those of zinc, so that the free copper(II) ion/free zinc(II) ion ratio is very much less than the total copper/total zinc ratio. Under the specified conditions, complex-formation with copper(II) is virtually complete whereas about 8% of the zinc remains free.

The present calculations represent a first step towards the establishment of a model for metal-complex equilibria in blood plasma. They are necessarily crude but will be progressively refined by allowing for other metal ions and other ligands, including peptides, and also by the further study of mixed-complex-formation. This approach is also important to the study of the interaction of copper or zinc ions with proteins such as albumin, particularly as the concentrations of free metal ions are maintained under physiological conditions at low values by the metal-buffering capacity of the free amino acids that are ordinarily present in such solutions. Evidence has been presented (Sarkar & Wigfield, 1968) for an albumin-copper(II)-amino acid complex, but there is insufficient information

for these interactions to be interpreted quantitatively. Similarly, the presence of ligands such as organic and inorganic phosphorus compounds and carboxylic acids might be expected to significantly decrease the concentrations of free calcium and magnesium ions in biological solutions.

Our results are, in general, consistent with the findings of Neumann & Sass-Kortsak (1967) but direct comparison is not possible because these workers used for their measurements similar orders of concentration for albumin, copper(II) and individual amino acids. The distribution pattern of copper(II) in such a system would be expected to show quantitative differences from similar systems in which only traces of copper were present.

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