Stability Constants of Complexes of Zinc and Cobalt(II) Ions with some Histidine-containing Peptides

By **Raghunath P. Agarwal** and **Douglas D. Perrin,*** Medical Chemistry Group, The John Curtin School of Medical Research, Australian National University, Canberra, A.C.T., Australia, 2600

Equilibrium constants at 37 °C and 0·15M (KNO_3) are reported for zinc and cobalt(II) complexes of glycyl-L-histidine, glycyl-L-histidylglycine, L-histidylglycine, and carnosine from pH titration data, using a range of metal ion and ligand concentrations. Binuclear complexes are major species in solutions containing zinc ions and glycylhistidine. Related mixed complexes are formed in ternary mixtures of zinc ion, histidine, and glycylhistidine. Mixed-ligand complexes of the type MAL are also formed by zinc and cobalt(II) with histidine (HA) and the peptides. A comparison is made of the zinc-complexing abilities of the peptides in competition with α-amino-acids under physiological conditions.

ALTHOUGH there is an extensive literature on metal-binding by α-amino-acids, few stability constants are available for complexes of metal ions with simple peptides. In particular there has been little quantitative information on metal-binding by histidine-containing peptides notwithstanding their importance as model systems for proteins in which histidyl residues serve as binding sites for metal ions. Most of the metal–peptide studies have dealt only with the acidity constants for the metal ion-promoted loss of peptide hydrogens from the complexes.

Recent papers have reported stability constants for complexes of copper(II) ions with glycylhistidine,[1] histidylglycine,[1,2] glycylhistidylglycine,[1] histidylglycylglycine,[2] and carnosine (β-alanyl-L-histidine).[1] Results, based on the analysis of a single titration curve for each metal ion with each ligand, have also been published for iron(II), cobalt(II), nickel, copper(II), and zinc with histidylglycine and histidylglycylglycine.[3] Earlier, titration curves of equimolar mixtures of 12 kinds of metal ions with carnosine [4] and of solutions containing high ratios of peptide to metal ion [5] were analysed to afford stability constants of the resulting complexes.

We now report results of a detailed potentiometric study of complex formation by zinc and cobalt(II) ions with four histidine-containing peptides, singly and in the presence of histidine. In seeking to evaluate the relevant stability constants we have followed the procedure described earlier.[1]

EXPERIMENTAL

Materials.—Stock solutions of cobalt(II) nitrate (B.D.H. AnalaR) and zinc nitrate (B.D.H. Lab.R., recrystallised from water), acidified with known concentrations of nitric acid, were standardised against EDTA.[6] All other reagents were as previously described.[1]

Methods.—Potentiometric titrations [at 37 °C and $I = 0{\cdot}15$ mol l^{-1} (KNO_3)] and the resulting computations followed established practice,[1] but experimental difficulties limited the accessible pH ranges. In most of the zinc-containing solutions where total zinc and peptide concentrations were approximately equal, hydrolytic precipitation (as indicated by a steady downward drift in the pH meter readings) occurred during titrations when the pH was raised to *ca.* $7 \pm 0{\cdot}3$. Because of the sensitivity of the cobalt(II) complexes to oxidation by traces of oxygen, the nitrogen used to purge the titration cell was given a prior passage through chromous chloride solution. Even so, oxidation occurred above *ca.* pH 8 and titration data for this region had to be rejected. Equilibrium constants for zinc and cobalt(II) ions with histidine, needed in seeking evidence of ternary complex formation of the metal ions with peptide and histidine, were also obtained from pH titration measurements. Ranges of metal ion concentrations, ligand concentrations, and pH values are summarised in Table 1.

For titrations in mixed-ligand systems, approximately

[1] R. P. Agarwal and D. D. Perrin, *J.C.S. Dalton*, 1975, 268.
[2] H. Aiba, A. Yokoyama, and H. Tanaka, *Bull. Chem. Soc. Japan*, 1974, **47**, 136.
[3] A. Yokoyama, H. Aiba, and H. Tanaka, *Bull. Chem. Soc. Japan*, 1974, **47**, 112.
[4] G. R. Lenz and A. E. Martell, *Biochemistry*, 1964, **3**, 751.
[5] R. B. Martin and J. T. Edsall, *J. Amer. Chem. Soc.*, 1960, **82**, 1107.
[6] G. Schwarzenbach and H. Flaschka, 'Complexometric Titrations,' Methuen, London, 2nd edn., 1969.

equimolar concentrations of the reactants were used, in the range 1—5 mM. All titration data have been deposited as a Supplementary publication [SUP 21294 (31 pp.)].*

RESULTS AND DISCUSSION

A computer-based approach [1] was followed in obtaining the constants listed in Tables 2 and 3. These constants reproduce the titration data for each metal ion–

TABLE 1

Summary of titrations used in obtaining stability constants

Metal ion	M_T/mM	Ligand	L_T/mM	pH Range
Zn	1·0—5·0	Gly·His	2·1—10	6·3—9·0
Co^{II}	0·9—5·0	Gly·His	1·8—10	5·9—8·1
Zn	0·5—2·5	Gly·His·Gly	0·6—5·0	5·9—9·4
Co^{II}	0·5—2·5	Gly·His·Gly	0·5—5·1	6·0—7·9
Zn	0·5—5·0	His·Gly	1·0—5·6	4·9—7·5
Co^{II}	1·0—2·5	His·Gly	2·2—5·2	5·0—6·9
Zn	1·0—5·0	Carn	2·0—10	6·0—7·6
Co^{II}	2·5—5·0	Carn	2·6—9·5	6·1—7·7
Zn	2·5—5·0	His	5·1—10	5·1—7·1
Co^{II}	2·5—5·0	His	2·5—10	4·9—6·6

TABLE 2

Equilibrium constants of zinc complexes at 37 °C in I = 0·15 mol l^{-1} (KNO_3) solutions

Equilibrium	log K ± s.d.
(1) Glycylhistidine (pK_a values 2·65, 6·61, 7·97) [1]	s.d.t. 0·0016 ml
$Zn + L \rightleftharpoons ZnL$	3·65 ± 0·02
$Zn + 2L \rightleftharpoons ZnL_2$	6·89 ± 0·04
$2ZnL \rightleftharpoons Zn_2L_2$	3·30 ± 0·04
$Zn_2L_2 \rightleftharpoons Zn_2L(L - H) + H$	−7·39 ± 0·07
$Zn_2L(L - H) \rightleftharpoons Zn_2(L - H)_2 + H$	−6·19 ± 0·07
(2) Gly·His·Gly (pK_a values 3·03, 6·36, 7·68) [1]	s.d.t. 0·0021 ml
$Zn + L \rightleftharpoons ZnL$	2·90 ± 0·12
$Zn + L \rightleftharpoons Zn(L - H) + H$	−2·55 ± 0·01
$Zn(L - H) \rightleftharpoons Zn(L - 2H) + H$	−9·69 ± 0·03
(3) Histidylglycine (pK_a values 2·32, 5·39, 7·15) [1]	s.d.t. 0·0017 ml
$Zn + L \rightleftharpoons ZnL$	4·25 ± 0·01
$Zn + 2L \rightleftharpoons ZnL_2$	8·46 ± 0·01
$Zn + HL \rightleftharpoons ZnHL$	2·37 ± 0·03
(4) Carnosine (pK_a values 2·64, 6·58, 9·04) [1]	s.d.t. 0·0012 ml
$Zn + L \rightleftharpoons ZnL$	3·86 ± 0·01
$Zn + HL \rightleftharpoons ZnHL$	2·18 ± 0·01
(5) Histidine (pK_a values 2·48 ± 0·01, 5·87 ± 0·01, 8·76 ± 0·01)	s.d.t. 0·0023 ml
$Zn + L \rightleftharpoons ZnL$	6·22 ± 0·01
$Zn + 2L \rightleftharpoons ZnL_2$	11·49 ± 0·02
$Zn + HL \rightleftharpoons ZnHL$	2·08 ± 0·10

peptide system, to within the stated computed standard deviations in titre (s.d.t.).

Glycyl-L-histidine.—Atomic models (Courtauld and Leybold) suggested that in any structure for ZnL, where L is the glycylhistidine anion, the metal ion is bonded through the peptide nitrogen, a carboxylate oxygen, and either the primary amino- or the imidazole-1-nitrogen. Strain precludes bonding through both of the latter. As log K_1 (3·56) and log β_2 (6·89) for the species ZnL and ZnL_2 are comparable with values (3·24 [7] and 5·88 [7]) for the zinc–glycylglycine complexes and are much greater than for the zinc complexes of acetylhistidine (2·50 and 4·80 at 25 °C [8]), bonding through the amino-group appears to be the more likely. The comparable constants for CoL and CoL_2 (3·37 and 6·28) are consistent with the same binding sites.

In the ligand HL the primary amino-nitrogen atom is protonated, so that in CoHL the bonding may be through the imidazole-1-nitrogen atom. Consistent with this, the logarithm of the stability constant of CoHL (2·23) is almost the same as for the cobalt complex of acetylhistidine (2·35 at 25 °C [8]). Against this, however, a similar value would be expected if binding in CoHL is the same as for CoL and the imidazole nitrogen is protonated. The

TABLE 3

Equilibrium constants of cobalt(II) complexes at 37 °C in I = 0·15 mol l^{-1} (KNO_3) solutions

Equilibrium	log K ± s.d.
(1) Glycylhistidine	s.d.t. 0·0018 ml
$Co + L \rightleftharpoons CoL$	3·37 ± 0·01
$Co + 2L \rightleftharpoons CoL_2$	6·28 ± 0·03
$Co + HL \rightleftharpoons CoHL$	2·23 ± 0·03
$CoL \rightleftharpoons Co(L - H) + H$	−7·19 ± 0·02
(2) Gly·His·Gly	s.d.t. 0·0017 ml
$Co + L \rightleftharpoons CoL$	3·17 ± 0·04
$CoL \rightleftharpoons Co(L - H) + H$	−6·09 ± 0·05
$Co(L - H) + L \rightleftharpoons CoL(L - H)$	2·54 ± 0·06
(3) Histidylglycine	s.d.t. 0·0019 ml
$Co + L \rightleftharpoons CoL$	4·54 ± 0·01
$Co + 2L \rightleftharpoons CoL_2$	8·16 ± 0·02
$Co + HL \rightleftharpoons CoHL$	2·17 ± 0·07
(4) Carnosine	s.d.t. 0·0014 ml
$Co + L \rightleftharpoons CoL$	3·22 ± 0·01
$Co + HL \rightleftharpoons CoHL$	1·98 ± 0·02
(5) Histidine	s.d.t. 0·0019 ml
$Co + L \rightleftharpoons CoL$	6·56 ± 0·01
$Co + 2L \rightleftharpoons CoL_2$	11·82 ± 0·01

difference of 1·1 in the logarithms of the stability constants of CoL and CoHL could be attributed to electrostatic effects.

Initial attempts to refine constants for mononuclear zinc complexes with glycylhistidine from potentiometric titration data revealed strong dependence of the values on zinc ion concentration. This difficulty was overcome, and good constants were obtained, when the data were treated on the assumption that binuclear zinc complexes were formed. We suggest that these species arise by dimerisation of two ZnL complexes by cross-co-ordination through their imidazole nitrogen atoms and that they are analogous to the binuclear complexes formed by copper(II) with 2,7-diaminosuberic acid,[9] cystine,[10] and carnosine.[11] In neutral or slightly alkaline solution the species $Zn_2(L - H)_2$ predominates. The pK_a values of 6·19 and 7·39 are attributed to ionisation of peptide hydrogens. They are to be compared with values of 6·50 and 7·10 obtained by Martin and Edsall [8] from titrations of high equimolar concentrations of zinc ion

* For details of the Supplementary publications scheme, see *J.C.S. Dalton*, 1974, Index issue, 'Notice to Authors No. 7.'

[7] R. P. Agarwal and D. D. Perrin, *Trans. Royal Inst. Technol. Stockholm (Pure and Applied Chem.)*, 1972, **34**, 387.

[8] R. B. Martin and J. T. Edsall, *J. Amer. Chem. Soc.*, 1960, **82**, 1107.

[9] C. J. Hawkins and D. D. Perrin, *Inorg. Chem.*, 1963, **2**, 839.

[10] C. J. Hawkins and D. D. Perrin, *Inorg. Chem.*, 1963, **2**, 843.

[11] H. C. Freeman and J. T. Szymanski, *Acta Cryst.*, 1967, **22**, 406.

and peptide. No evidence of binuclear complex formation by copper(II) and glycylhistidine was found earlier,[1] possibly because bonding in CuL is through three nitrogen atoms [1] and any dimerisation would depend only on binding through the carboxylate ions. Nevertheless, in crystalline copper glycylhistidine sesquihydrate the three nitrogen atoms are all at approximately the same distance from the central copper ion and charge neutralisation is achieved by a carboxy oxygen from another molecule of ligand.[12]

Glycyl-L-histidylglycine.—Values of log K_1 for zinc (2·90) and cobalt(II) (3·17) complexes of Gly·His·Gly are very similar to the value of log K_1 (3·00 [5]) for zinc ion with triglycine. Non-involvement of the imidazole nitrogen in bonding by Gly·His·Gly is suggested.

The first pK values for the 1 : 1-zinc (5·45) and -cobalt(II) (6·09) complexes are attributed to ionisation of the peptide hydrogen atom. The corresponding lowering of the pK value for the 1 : 1-copper(II) complex (3·20) relative to the glycylhistidine complex (4·14 [1]) was explained by the acid-strengthening effect on the peptide hydrogen in the parent ligand when the carboxylate ion was replaced by an amide group.[1] A similar effect seems likely in the zinc and cobalt(II) complexes. The second pK of the zinc–Gly·His·Gly complex (at 9·69) is comparable with the second pK of the copper complex (9·01 [1]). The latter was suggested to be due to proton loss from a water molecule co-ordinated to the complexed metal ion [1] or to the ionisation of the pyrrole hydrogen of the imidazole ring.[8]

L-Histidylglycine.—The logarithms of the stability constants of the protonated species ZnHL (2·37) and CoHL (2·17) are almost the same as for the corresponding cobalt(II) complex of glycyl-L-histidine (2·23). In this case, however, there is little doubt that the site of protonation is the imidazole nitrogen, with the amino-nitrogen co-ordinated to the metal ion. The alternative possibility would require the formation of a 7-membered chelate ring.

Agreement of values of log K_1 for ZnL (4·25) and of log β_2 for ZnL_2 (8·46) with constants for the corresponding complexes for histidine methyl ester [4·46 and 8·66 at 25 °C, I = 0·16 mol l^{-1} (KNO_3) (ref. 13)] suggests that bonding is principally through the imidazole and amino-nitrogens, although some contribution from the peptide group cannot be excluded. A similar comment applies to the cobalt(II) complexes [4·54 and 8·16, compare 4·24 and 7·36 at 25 °C, I = 0·25 mol l^{-1} (KCl) (ref. 14)].

Carnosine.—Our values for the constants for ZnHL and CoHL (2·18 and 1·98) lie in the same range as those of the other histidine-containing peptides. They are appreciably less than the single constants [3·39 and 3·69 at 25 °C and I = 0·1 mol l^{-1} (KNO_3)] previously [4] reported. The stability constants of ZnL and CoL are comparable with values for the corresponding glycylhistidine complexes (log K_1 = 3·86 and 3·22; compare 3·56 and 3·37, respectively), and we suggest that the same sites are involved in metal co-ordination (except that replacement of the glycyl moiety by β-alanyl leads to one of the chelate rings being 6- instead of 5-membered).

Mixed-ligand Species with Histidine.—Titration curves for systems containing histidine with zinc or cobalt(II) ions were substantially in agreement with earlier [15] results but the computed stability constants are lower because of differences in the values used for the ionisation constants of histidine.

Comparison of the potentiometric titration curves of solutions containing zinc or cobalt(II) ions with glycylhistidine (or histidylglycine) and histidine, singly and in admixture, showed that mixed-ligand complexes were also formed. To account for the shapes of the titration curves of the mixed solutions stability constants additional to those given in Tables 2 and 3 were needed. These are listed in Table 4.

TABLE 4

Equilibrium constants of mixed-ligand complexes of zinc and cobalt(II) ions with histidine (HA) and glycylhistidine or histidylglycine (HL) at 37 °C and I = 0·15 mol l^{-1} (KNO_3)

Peptide	Equilibrium	log K + s.d.
Gly·His	$Zn + A + L \rightleftharpoons ZnA(L - H) + H$	4·70 ± 0·03
	$ZnA(L - H) \rightleftharpoons ZnA(L - 2H) + H$	−8·01 ± 0·03
	$2\,Zn + A + L \rightleftharpoons Zn_2A(L - H) + H$	10·04 ± 0·03
	$Zn_2A(L - H) \rightleftharpoons Zn_2A(L - 2H) + H$	−6·68 ± 0·03
Gly·His	$Co + A + L \rightleftharpoons CoAL$	9·36 ± 0·02
His·Gly	$Zn + A + L \rightleftharpoons ZnAL$	10·12 ± 0·01
	$2\,Zn + A + L \rightleftharpoons Zn_2AL$	12·64 ± 0·10
His·Gly	$Co + A + L \rightleftharpoons CoAL$	10·50 ± 0·01
	$CoAL \rightleftharpoons CoA(L - H) + H$	−7·98 ± 0·04

Titration curves for the zinc and cobalt(II), histidine, and Gly·His·Gly systems, computed on the assumption that no mixed-ligand complexes were formed, were almost identical with experiment. Because of problems due to sparing solubility, no attempt was made to study mixed-complex formation in mixtures of carnosine and histidine.

Between pH 5·3 and 7·3 the binuclear species $Zn_2A(L - H)$ and $Zn_2A(L - 2H)$ are the major complexes present in zinc solutions containing equimolar concentrations of histidine and glycylhistidine. They are probably structurally similar to the corresponding binuclear bis(glycylhistidine)zinc complexes. Stability constants of the ternary complexes of zinc or cobalt(II) with histidine and Gly·His or His·Gly are about what would be expected from the stability constants of the relevant binary complexes. Thus log β for CoHis(Gly·His) is 9·36, whereas the mean of log β_2 for $Co(Gly·His)_2$ and $Co(His)_2$ is $\frac{1}{2}(6·28 + 11·82) = 9·05$.

The effectiveness of the peptides as chelating agents under physiological conditions was examined by computing the equilibrium distribution of copper(II) and zinc ions in an amino-acid model for blood plasma,[16] as was

[12] J. F. Blount, K. A. Fraser, H. C. Freeman, J. T. Szymanski, and C. H. Wang, *Acta Cryst.*, 1967, **22**, 396.
[13] H. L. Conley and R. B. Martin, *J. Phys. Chem.*, 1965, **69**, 2923.
[14] A. C. Andrews and D. M. Zebolsky, *J. Chem. Soc.*, 1965, 742.
[15] D. D. Perrin and V. S. Sharma, *J. Chem. Soc.* (*A*), 1967, 724.
[16] P. S. Hallman, D. D. Perrin, and A. E. Watt, *Biochem. J.*, 1971, **121**, 549.

done previously for copper(II).[1] When the concentration of each peptide in turn was set equal to the average histidine content (74 μM), differences in binding ability became apparent. Carnosine–zinc complexes accounted for less than 0·2% of the total zinc. With glycylhistidine, mixed-ligand complexes of peptide and histidine were major species. Computed distributions of zinc ions, as a percentage of total zinc present, for glycylhistidine complexes were: ZnHis(L — H), 24; ZnHis(L — 2H), 6; Zn_2His(L — H), 5; Zn_2His(L — 2H), 28%. For histidylglycine, 5% of the zinc ions were present as ZnHisL, while the only important Gly·His·Gly complex was Zn(L — H) which accounted for 9% of the total zinc. The earlier computed distribution of copper(II) ions among the amino-acids and peptides [1] was only marginally affected by allowing also for zinc ions.

[4/2296 *Received, 5th November,* 1974]