# Effect of Mixed-ligand Complex Formation on the Ionization of the Pyrrole Hydrogens of Histamine and Histidine

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Equilibrium studies have been carried out by pH titrimetry to determine the stability constants of the parent complexes of $Cu^{II}$, $Ni^{II}$, and $Zn^{II}$ with histamine and histidine, and of the mixed-ligand complexes of these with glycine, ethylenediamine, 4,5-dihydroxybenzene-1,3-disulphonate, and 2,2'-bipyridyl. From titrations in an alkaline medium it is concluded that nickel(II) and zinc(II) ions do not affect the ionization of the pyrrole hydrogen significantly compared to the free ligand, but $Cu^{II}$ decreases the relevant p$K$ values in the parent complexes by about three orders of magnitude. As a consequence of charge-transfer processes, the deprotonation can also be influenced significantly by the formation of mixed-ligand complexes.

DURING the past 20 years widespread investigations have been performed on the complex-forming properties of imidazole derivatives {primarily histamine [4-(2-aminoethyl)imidazole] and histidine (His)} which are of outstanding biological importance.[1] Relatively few experiments have been carried out, however, on the mixed-ligand complexes of histamine and His.[2] Similarly, those papers which are connected with the metal ion-

[1] R. J. Sundberg and R. B. Martin, *Chem. Rev.*, 1974, **74**, 471.
[2] R. P. Martin, M. M. Petit-Ramel, and J. P. Scharff, 'Mixed Ligand Metal–Ion Complexes of Amino Acids and Peptides,' in 'Metal Ions in Biological Systems,' vol. 2, ed. H. Sigel, Marcel Dekker, New York, 1973.

induced ionization of pyrrole hydrogen are in part insufficient or contradictory.[3,4]

The mixed-ligand complexes of histamine have primarily been studied by Perrin and his co-workers [5,6] who found there is a considerable stability increase in the copper(II)– and nickel(II)–histamine–serine systems. In connection with the complexes of $Cu^{II}$, we have pointed out [7-9] that histamine primarily favours co-ordination of amino-acids. This was explained by the interaction between the copper(II) ion and the π-electron for the free ligand. Examinations of this type were first carried out for histamine and His by Morris and Martin [16] and Aiba *et al.*[17] Bogges and Martin [18] pointed out that for imidazole derivatives in 50% ethanol the ionization is promoted by the 3*d* transition metals according to the sequence $Fe^{II} > Cu^{II} \sim Co^{II} > Zn^{II} > Ni^{II}$. On the other hand, certain workers [3,4] obtained different values for the deprotonation of the NH nitrogen in the presence of $Zn^{II}$. This difference may originate from the fact that, in terms of equilibria, it is very difficult to

TABLE 1

Stability constants, $\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$, of parent complexes of histamine and histidine at 25 °C and $I = 0.2$ mol $dm^{-3}$ (KCl) [a]

| System | $\log\beta_{110}$ | $\log\beta_{120}$ | $\log\beta_{130}$ | $\log\beta_{111}$ | $\log\beta_{121}$ | $\log\beta_{122}$ | $\log\beta_{22-2}$ | $\log\beta_{11-1}$ | $\log(K_1/K_2)$ [b] |
|---|---|---|---|---|---|---|---|---|---|
| Copper(II)–histamine | 9.58 | 16.06 | | | 21.79 | | 7.0 | 1.2 | 3.10 |
| Nickel(II)–histamine | 6.85 | 11.93 | 15.13 | 11.56 | | | | | 1.77 |
| Zinc(II)–histamine | 5.56 | 10.29 | | 11.78 | | | | −2.83 | 0.83 |
| Copper(II)–histidine | 10.04 | 17.82 | | 14.07 | 23.63 | 27.13 | 8.0 | 1.5 | 2.26 |
| Nickel(II)–histidine | 8.52 | 15.30 | | | | | | | 1.74 |
| Zinc(II)–histidine | 6.31 | 11.84 | | 11.37 | 17.55 | | | | 0.78 |

[a] Standard deviations of all quantities given = ±0.02. [b] $\log K_1 = \log\beta_{110}$ and $\log K_2 = \log\beta_{120} - \log\beta_{110}$.

TABLE 2

Stability constants of parent complexes of glycine (Gly), ethylenediamine (en), 4,5-dihydroxybenzene-1,3-disulphonate (dbds), and 2,2′-bipyridyl (bipy). Details as in Table 1

| System | $\log\beta_{110}$ [a] | $\log\beta_{120}$ [a] | $\log\beta_{130}$ [a] | $\log(K_1/K_2)$ | Ref. |
|---|---|---|---|---|---|
| Copper(II)–Gly | 8.07 | 14.84 | | 1.30 | 8 |
| Copper(II)–en | 10.57 | 19.68 | | 1.46 | 8 |
| Copper(II)–dbds | 13.73 | 25.08 | | 2.38 | *b* |
| Copper(II)–bipy | 8.50 | 13.65 | 16.95 | 3.35 | *c* |
| Nickel(II)–Gly | 5.65 | 10.40 | | 0.90 | *d* |
| Nickel(II)–en | 7.36 | 13.52 | 17.78 | 1.20 | *b* |
| Zinc(II)–Gly | 4.84 | 9.02 | | 0.66 | *b* |
| Zinc(II)–en | 5.78 | 10.73 | | 0.83 | *b* |

[a] Standard deviations = ±0.02. [b] This work. [c] H. Irving and D. H. Mellor, *J. Chem. Soc.*, 1962, 5222. [d] I. Sóvágó, A. Gergely, and J. Posta, *Acta Chim. Acad. Sci. Hung.*, 1975, **85**, 153.

system of the imidazole ring, in agreement with the findings of Sigel and his co-workers [10,11] on complexes with other imidazole and pyridine derivatives. Many workers have studied the interaction between transition-metal ions and His. Of the mixed-ligand complexes, however, mainly copper(II)–histidine–amino-acid systems have been investigated.[12-14] In order that conclusions of a more general validity may be drawn, it is necessary to extend the studies to other mixed-ligand complexes containing His.

It was observed earlier [1,15] that in certain transition-metal complexes of imidazole derivatives the pyrrole hydrogen may undergo ionization at a lower pH than distinguish between the formation of hydroxo-complexes and the deprotonation process in alkaline media.

Thus, a critical revision of the available data appears to be justified. In addition, in real biological systems histamine and His are primarily to be found in the form of mixed-ligand complexes,[19] where most of the earlier studies connected with the ionization related to the parent complexes. Therefore, we have studied the parent and mixed-ligand complexes of $Cu^{II}$, $Ni^{II}$, and $Zn^{II}$ with histamine and His over a wide pH range, with a view to determining the stability and deprotonation constants.


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[18] R. K. Bogges and R. B. Martin, *Inorg Chem.*, 1974, **13**, 1525.
[19] D. D. Perrin and R. P. Agarwal, 'Multimetal–Multiligand Equilibria: A Model for Biological Systems,' in 'Metal Ions in Biological Systems,' vol. 2, ed. H. Sigel, Marcel Dekker, New York, 1973.

## EXPERIMENTAL

The chemicals used were Reanal products of the highest analytical purity, which were further purified by recrystallization from ethanol–water or acetone–water. L-Histidine was used in all the experiments. Determinations of the concentrations of the stock solutions, and also the pH-metric examinations and their evaluation, were performed as described previously.[8,20]

Ionization of the pyrrole hydrogen in the copper(II)–histamine and –histidine systems was also followed spectrophotometrically in the visible range using a Beckman Acta M IV spectrophotometer. The zinc(II)–histidine system was studied on a JEOL MH-100 n.m.r. instrument in aqueous solutions with a histidine concentration of 0.2 mol $dm^{-3}$ at a metal ion : ligand ratio of 1 : 2.5.

## RESULTS AND DISCUSSION

The equilibrium constants of the parent complexes are listed in Table 1. It may be concluded that, in agreement with other observations,[21,22] histidine can co-ordinate both as an amino-acid and like histamine in the copper(II)– and zinc(II)–histidine systems. In the nickel(II)–histidine system, on the other hand, since a species of composition $ML_3$ (L = amino-acid) is not formed, all three donor groups of the ligand participate in the co-ordination.[23,24] (The parent complexes of histamine with $Ni^{II}$ and $Cu^{II}$ were described in detail earlier.[8]) In the zinc(II)–histamine system, in contrast to the literature data, we detected the formation of a hydroxo-complex of composition $[ZnL(OH)]^+$. Above pH *ca.* 8.5 an increase in the quantity of hydroxo-complexes leads to precipitate formation. In the zinc(II)–histidine system, however, there is no precipitation even at higher pH.

Mixed-ligand complex formation was examined for all the three metal ions, using histamine or His as ligand L, and glycine (Gly), ethylenediamine (en) [and in the case of $Cu^{II}$, 2,2′-bipyridyl (bipy), or 4,5-dihydroxybenzene-1,3-disulphonate (dbds)] as ligand L′; the mixed-ligand systems containing both histamine and His were also studied. The stability constants of the parent complexes of ligands L′ are given in Table 2, and the equilibrium data on the mixed-ligand complexes in Table 3.

A comparison of the data in Table 3 leads to the following major findings. (*i*) Our earlier finding [9] in connection with the mixed-ligand complexes of diamines, that the stability of a mixed-ligand complex is higher the greater is the difference between the log $(K_1/K_2)$ values for the parent complexes, also holds as regards the cases of histamine and His. Because of the change in charge, however, the stabilization of the glycine-containing mixed-ligand complexes is always higher than that of the corresponding ethylenediamine complex. At the same time, it is noteworthy that for $Ni^{II}$ and $Zn^{II}$ there is a destabilization of the relevant histamine–ethylenediamine complexes. This can be interpreted in that the stability-increasing effect of the π-electron system of imidazole appears only in the complex $[CuL]^{2+}$, but for steric reasons not in $[CuL_2]^{2+}$,[7] which at the same time leads to extensive mixed-ligand complex formation. In contrast, for $Ni^{II}$ and $Zn^{II}$ the formation process is favoured for both the $[ML]^{2+}$ and $[ML_2]^{2+}$ parent complexes and thus the formation of mixed-ligand complexes is less important.

(*ii*) In the mixed-ligand complexes of His containing $Cu^{II}$, in addition to the species [CuL(L′)], a complex of

TABLE 3

Stability constants, $\beta_{pqr} = [M_pL_qL'_rH_s]/[M]^p[L]^q[L']^r[H]^s$, of mixed-ligand complexes of histamine and histidine at 25 °C and $I$ = 0.2 mol $dm^{-3}$ (KCl)

| M | L | L′ | log $\beta_{1110}$ [a] | $\Delta\log\beta_{1110}$ [b] | $\Delta\log K$ [c] | log $\beta_{1111}$ [a] | $\Delta\log\beta_{111}$ |
|---|---|---|---|---|---|---|---|
| $Cu^{II}$ | Histamine | Gly | 17.00 | 1.25 | 0.65 | | |
| | Histamine | en | 18.66 | 0.49 | 1.49 | | |
| | Histamine | His | 18.21 | 0.47 | 1.41 | 23.38 | 1.48 |
| | Histamine | bipy | 15.40 | 0.24 | 2.68 | | |
| | Histamine | dbds | 22.55 | 1.68 | 0.76 | | |
| | His | Gly | 17.43 | 0,80 | 0.68 | 21.44 | 0.15 |
| | His | en | 19.46 | 0.41 | 1.15 | 24.21 | 0.50 |
| | His | bipy | 16.84 | 0.80 | 1.70 | 22.07 | 1.38 |
| | His | dbds | 22.60 | 0.85 | 1.17 | 26.88 | 0.47 |
| $Ni^{II}$ | Histamine | Gly | 11.77 | 0.30 | 0.73 | | |
| | Histamine | en | 12.89 | −0.14 | 1.32 | | |
| | Histamine | His | 14.27 | 0.35 | 1.10 | | |
| | His | Gly | 13.43 | 0.28 | 0.74 | | |
| | His | en | 14.84 | 0.13 | 1.04 | | |
| $Zn^{II}$ | Histamine | Gly | 9.97 | 0.01 | 0.43 | | |
| | Histamine | en | 10.60 | −0.21 | 0.74 | | |
| | Histamine | His | 11.48 | 0.11 | 0.39 | | |
| | His | Gly | 10.89 | 0.16 | 0.26 | | |
| | His | en | 11.65 | 0.06 | 0.44 | | |

[a] Standard deviation = ±0.05. [b] $\Delta\log\beta_{1110} = \log\beta_{1110}^{expt} - \frac{1}{2}(\log\beta_{1200} + \log\beta_{1020} + \log 4)$. [c] $\Delta\log K = \log K_M^{ML} - \log K_{ML'}^{MLL'} = \log K_M^{ML'} - \log K_{ML}^{MLL'}$.

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composition [CuL(L')H] is also formed. In the latter complex the mode of co-ordination of His is amino-acid-like, as in the protonated parent complexes. Earlier findings [9,11] indicated that the co-ordination of amino-acids is primarily promoted by ligands containing an *N*-donor atom in the aromatic ring. Accordingly, the proportion of protonated mixed-ligand complexes is highest in the copper(II)–histamine–His and copper(II)–bipy–His systems, and least in the copper(II)–Gly–His system.

(*iii*) As regards the mixed-ligand complexes of composition [CuL(L')], the largest stability increase occurs in the case of L' = dbds. For the histamine-containing mixed-ligand complexes the stability increase is lowest in the copper(II)–histamine–bipy and copper(II)–histamine–en systems. These data again show that aromatic amines primarily promote the co-ordination of ligands which also contain an *O*-donor atom.[11]

For the determination of the ionization of the pyrrole hydrogen the parent and mixed-ligand complexes were titrated to pH *ca.* 12. Evaluation of the alkaline regions of the titration data yields the following findings. (*a*) Because of precipitate formation, the nickel(II)–histamine system can be examined only up to pH *ca.* 11.5; however, further consumption of base can be neglected. Accordingly, the titration curves of systems with metal : ligand ratios greater than 1 : 2 are identical with the curves for solutions containing an excess of ligand, but not containing metal ion.

(*b*) A significant alkali-consuming process begins at pH *ca.* 8 in the zinc(II)–histamine system, and at pH *ca.* 10 in the zinc(II)–His system. On the basis of literature analogies relating to other zinc(II) complexes, this can most probably be explained by the formation of hydroxo-complexes. This assumption was confirmed by n.m.r. study of the zinc(II)–His system. The chemical shifts referring to the $C^2$ and $C^5$ protons of the imidazole ring in the cases of His and the zinc(II)–His system are shown in Figure 1, as functions of pH.

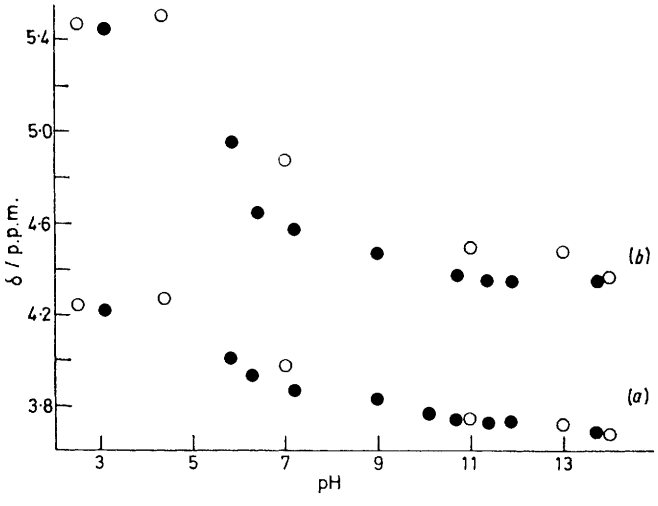


FIGURE 1 Chemical shifts of the $C^2$ (*a*) and $C^5$ protons (*b*) of the histidine imidazole ring as functions of pH: (○) $c_L = 0.2$ mol dm$^{-3}$; (●) $c_L = 0.2$, $c_{Zn} = 0.08$ mol dm$^{-3}$

From Figure 1 it may be stated that, in the interval $4 < pH < 10$, both the deprotonation of the histidine and the formation of complexes $[ZnL]^+$ and $[ZnL_2]$ result in considerable changes in the positions of the $C^2$ and

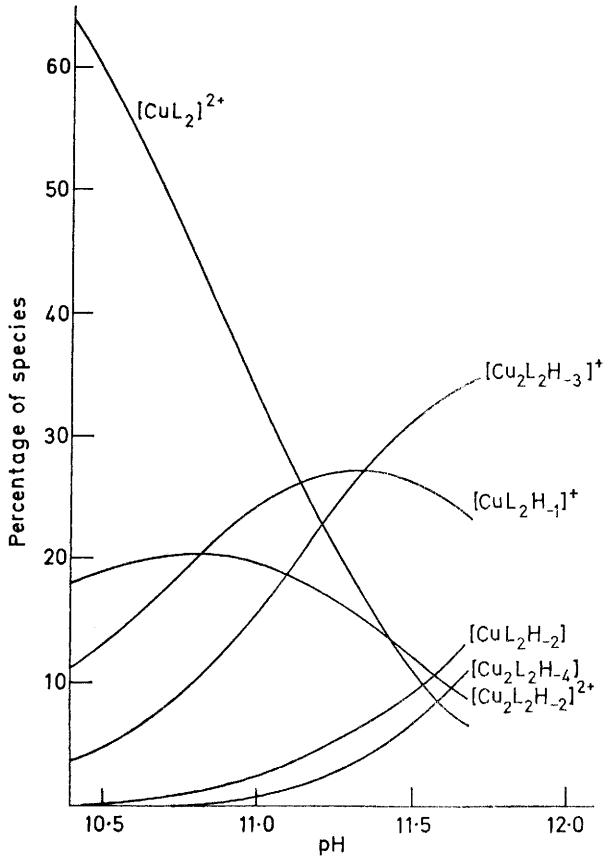


FIGURE 2 Concentration distribution of the complexes formed in the copper(II)–histamine system in an alkaline medium: $c_{Cu} = 5 \times 10^{-3}$, $c_L = 0.01$ mol dm$^{-3}$

$C^5$ proton signals. However, the alkali-consuming process at pH >10 no longer causes a further change in the n.m.r. signal. This is in agreement with the insignificant effect of the binding of the hydroxyl groups on the signals of the $C^2$ and $C^5$ protons.

(*c*) In parent complexes of $Cu^{II}$ the alkali-consuming process similarly occurs at pH >10. At the same time, this also results in a shift of the visible spectrum towards higher energies: the absorption maximum of the copper(II)–His complex $[CuL_2]$ lies at 645 nm, but up to pH *ca.* 12 this decreases to 627 nm. It should also be noted that the process is reversible only in the event of the complete exclusion of $O_2$. On standing in air the solutions undergo a very rapid change in colour, for in alkaline media the copper(II)-catalysed oxidations of histamine and particularly His (processes not clarified in detail) are greatly accelerated. This experimental fact confirms that the alkali-consuming process is accompanied by a change in the structure of the ligand, *i.e.* by the ionization of the pyrrole hydrogen. This is also supported by the fact that the equilibrium evaluation of the systems is possible only by the simultaneous

assumption of deprotonated, $[CuL_2H_{-1}]^+$ and $[CuL_2H_{-2}]$, hydroxo-, $[Cu_2L_2H_{-2}]^{2+}$, and deprotonated hydroxo-complexes, $[Cu_2L_2H_{-3}]^+$ and $[Cu_2L_2H_{-4}]$. The p$K$ values calculated in this way are listed in Table 4. Figure 2

TABLE 4

Deprotonation constants of the NH group in copper(II) complexes of histamine and histidine at 25 °C, $I$ = 0.2 mol dm$^{-3}$ (KCl); p$K_w$ = 13.75

| Ligand (L) | Complex | p$K$ * |
|---|---|---|
| Histamine | $[CuL_2]^{2+}$ | 11.1 |
| | $[CuL_2H_{-1}]^+$ | 11.9 |
| | $[Cu_2L_2H_{-2}]^{2+}$ | 11.0 |
| | $[Cu_2L_2H_{-3}]^+$ | 12.1 |
| | $[CuL(L')]$ (L′ = $[dbds]^{2-}$) | 12.9 |
| | (L′ = bipy) | 10.0 |
| Histidine | $[CuL_2]$ | 11.4 |
| | $[CuL_2H_{-1}]^-$ | 12.9 |
| | $[Cu_2L_2H_{-2}]$ | 11.3 |
| | $[Cu_2L_2H_{-3}]^-$ | 11.9 |
| | $[CuL(L')]$ (L′ = $[Gly]^-$) | 11.2 |
| | (L′ = en) | 11.3 |
| | (L′ = $[dbds]^{2-}$) | 12.5 |
| | (L′ = bipy) | 10.6 |

* Standard deviation = ±0.1

presents the concentration distribution of the species formed, as a function of pH, for the copper(II)–histamine system.

From the data in Table 4 it may be stated that the presence of $Cu^{II}$ decreases the p$K$ value of the pyrrole hydrogen by about three orders of magnitude compared to the free ligand (p$K$ *ca.* 14.5). The decrease in p$K$ of the complexes of histamine is somewhat larger than in the case of His, which can presumably be explained by the different charge conditions.

(*d*) In the cases of L′ = Gly and en in mixed-ligand complexes of $Cu^{II}$ the p$K$ of the deprotonation process does not differ greatly from that of the parent complexes. This also showed that in these systems the increased stability of the mixed-ligand complex is primarily a consequence of steric factors. At the same time, the deprotonation process is appreciably suppressed in the copper(II)–dbds–histamine and copper(II)–dbds–His systems, while when L′ = bipy the p$K$ values decrease further by one order of magnitude compared to those for the parent complexes. This can be explained by the fact that the charge-transfer process in the direction O ⟶ $Cu^{2+}$ in the copper(II)–dbds system reduces the interaction between the $Cu^{II}$ and the π-electron system of the imidazole ring. Thus, ionization of the pyrrole hydrogen is inhibited relative to that in the parent complexes. Because of the back co-ordination between bipy and $Cu^{II}$, the electron shift is in the opposite direction, which promotes ionization. Consequently, as regards the deprotonation process, not only the metal ions but also the other ligands have significant effects, and these may result in further changes compared to the parent complexes, primarily by means of the various charge-transfer processes.

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