Equilibrium Studies of Binary and Mixed-ligand Complexes of Zinc(II) involving Imidazole, Histamine, and L-Histidine as Ligands

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Binary and mixed-ligand complexes formed by zinc(II) with imidazole, histamine, and L-histidine ligands (A) have been investigated by potentiometry in aqueous solution at 37 °C and I = 0.15 mol dm^{-3} ($Na[ClO_4]$) and the relevant stability constants are evaluated using advanced computer techniques. The results suggest that in the zinc(II)–imidazole, –histamine, and –L-histidine binary systems, the complex formation process is favoured by the π-electron system of the imidazole group in both the 1 : 1 and 1 : 2 complexes. It appears that the extra proton in the ZnAH histamine complex and ZnA_2H_2 and ZnA_2H L-histidine complexes resides on the primary amino-group of the respective ligands. The mixed-ligand systems, (i) zinc(II)–imidazole (A)–histamine (B), (ii) zinc(II)–imidazole (A)–L-histidine (B), and (iii) zinc(II)–histamine (A)–L-histidine (B) respectively showed the presence of the mixed species, (i) ZnAB and ZnA_2B, (ii) ZnAB, and (iii) ZnAB, ZnABH, and $ZnABH_2$. Formation of ZnA_2B in system (i) was found to be less favoured. All other mixed species do have marked stabilities. The probable site of protonation in ZnABH and $ZnABH_2$ species in system (iii) is discussed in terms of their stability constant data.

CONSIDERABLE attention has been paid in recent years to the investigation of the complex-forming properties of imidazole and its derivatives, histamine and histidine, because of their outstanding biological significance.[1-3] It has been shown [4] that the imidazole group of histidine is the most important binding site for zinc and other metal ions in the case of serum albumin. Albumin-bound zinc(II) has been found [4] to account for approximately 98% of the exchangeable fraction of zinc(II) in blood serum. This fraction is known [5,6] to be associated with the transport process of zinc(II). It has been suggested [1,7] that the zinc(II)–albumin interaction can be best described by either a simple 1 : 1 zinc(II)–imidazole interaction or a compound site involving an imidazole and a neighbouring carboxy group. The effectiveness of the imidazole group to act as a metal binding site has been attributed [1,2] to its great flexibility, its availability at physiological pH, and its capacity to form both σ and π bonds with metal ions. Thus, a number of reports [8,9] have been made on zinc(II) complexes of imidazoles in an effort to understand the nature of metal-ion complexation in biological systems. Appleton and Sarkar [10] explored the use of aquaimidazole complexes of zinc(II) as relevant models of the zinc(II) binding site in carbonic anhydrase. However, the tendency of the zinc(II)–imidazole system to polymerize precluded a thorough study. From subsequent studies on cobalt(II) and zinc(II) complexes of *N*-methylimidazole, it was concluded [11] that the occurrence of zinc(II) rather than cobalt(II) in native carbonic anhydrase is due to the stronger affinity of zinc(II) for imidazole. The co-ordination behaviour of zinc(II) with some histidine-containing tripeptides was recently investigated by Lakusta and Sarkar.[12] Reports are also available in the literature [13-16] on the binary and mixed-ligand complexes of zinc(II) containing histamine and L-histidine. By analyzing all these data, we thought it worthwhile to carry out a systematic study and a computer-based analysis of the experimental data of the binary and mixed-ligand complexes of zinc(II) involving imidazole, histamine, and L-histidine as ligands under biologically important conditions and the results obtained are reported in this paper.

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EXPERIMENTAL

Imidazole, histamine, L-histidine, and zinc(II) perchlorate were purchased from Fluka AG, Buchs, Switzerland. The concentration of the metal stock solution was determined by titration with ethylenediaminetetra-acetate using Eriochrome Black T indicator. All the solutions were prepared with doubly-distilled water. Acid-washed glassware and reagent grade chemicals were used throughout the work.

All titrations were carried out at 37 °C under nitrogen with 0.15 mol dm^{-3} $Na[ClO_4]$ as background electrolyte. The equipment and electrode standardization procedures have been described previously.[17] The acid dissociation constants for imidazole, histamine, and L-histidine were taken from our earlier work.[18] The complex formation constants were calculated from potentiometric titration curves obtained for a number of solutions with different metal: ligand ratios. Calculations were made with the aid of the MINIQUAD-75 computer program [19] on an IBM-370 computer. Various models were fitted to the data and the model selected was that which gave the best statistical fit, consistent with chemical logic, to the range of titration data without giving any systematic drifts in the magnitudes of various residuals, as described by Gans *et al.*[19] At high pH values, hydroxo-complexes were often present. Since these data could not be fitted satisfactorily to any simple model, points above the onset of a systematic drift in residuals were omitted. The results obtained are reported in Tables 1 and 2. The charges of all the complex species reported in this paper are omitted for clarity.

RESULTS AND DISCUSSION

Zinc(II)–Imidazole, –Histamine, and –L-Histidine Binary Systems.—In the zinc(II)–imidazole (A) binary system, the presence of four binary complexes (ZnA, ZnA_2, ZnA_3, and ZnA_4) in addition to HA was confirmed. The stability constant data reported in Table 1 for these complexes agree well with the literature data [8,9] after making allowances for the changes in experimental con-

ditions. However, unlike in the copper(II)–imidazole (A) system,[18] where the stepwise formation constants for the CuA, CuA_2, CuA_3, and CuA_4 complexes decrease from CuA to CuA_4, in the zinc(II)–imidazole (A) system, these constants are of comparable magnitude (Table 1). This clearly demonstrates that in the copper(II)–imidazole (A) system, the binding of the first imidazole by copper(II) is much stronger and this would decrease the affinity for imidazole of the other three available ligand positions around copper(II), while in the zinc(II)–imidazole (A) system the co-ordination of the first imidazole is comparatively weak and hence the binding tendency of the other three imidazole molecules would be enhanced. In other words, the copper(II)–imidazole system shows negative interactions between the imidazole ligands while the zinc(II)–imidazole system shows positive interactions. This is in agreement with the suggestion put forward by Gergely and Sovago [16] that the stability-enhancing effect of the π-electron system of the imidazole group appears only in the 1 : 1 and not in 1 : 2 complexes due to steric reasons in copper(II)–imidazole systems, while the complex formation process is favoured by the π-acceptor property of the imidazole group in both the 1 : 1 and 1 : 2 complexes in the zinc(II)–imidazole system. It may be noted that the stability constant value of 2.82 log units reported [20] for the binding of zinc(II) by human serum albumin compares favourably with the log K^{Zn}_{ZnA} value of 2.55 in the zinc(II)–imidazole (A) binary system (Table 1).

Although many workers have reported the formation equilibria involved in the zinc(II)–histamine (A) system, a more detailed study was recently carried out by Sovago *et al.*[14] at 25 °C and I = 0.20 mol dm^{-3} (KCl). Our results at 37 °C and I = 0.15 mol dm^{-3} ($Na[ClO_4]$) likewise showed the presence of three binary complexes (ZnAH, ZnA, and ZnA_2) in addition to HA and H_2A. It may be noted that Sovago *et al.*[14] could also detect the presence of $ZnAH_{-1}$ species in this system in the alkaline medium. The results in the present investigation on the zinc(II)–L-histidine (A) system contrast with the literature data with regard to the types of binary species detected. Though earlier studies [8] show only ZnA and ZnA_2 complexes in this system, recent studies by Sovago *et al.*[14] and Amico *et al.*[15] agree with the formation of ZnAH, ZnA, ZnA_2H, and ZnA_2. However, our results from the detailed titration studies show that the zinc(II)–L-histidine (A) system contains ZnA, ZnA_2H_2, ZnA_2H, and ZnA_2 as the major species in addition to HA, H_2A, and H_3A. The formation of ZnAH was also assumed in this system, but it was rejected in the final refinement of the computer-based analysis of the experimental data using MINIQUAD-75.

Zinc(II) forms a six-membered chelate ring in the ZnA histamine complex through imidazole- and primary amino-nitrogens. The higher log K^{Zn}_{ZnA} value of 6.41 for the ZnA L-histidine complex compared with that of 5.39 for the ZnA histamine complex indicates that L-histidine is tridentate in its ZnA complex. In the ZnAH histamine complex, the extra proton can attach either to the imidazole nitrogen or to the primary amino-group of histamine (A). In order to characterize the metal–ligand binding in this complex, the parameter log P was computed using equation (1). The value obtained (2.52) compares favourably with the log K^{Zn}_{ZnA} value of 2.55 in

$$\log P = \log \beta_{ZnAH} - \log \beta_{HA} \qquad (1)$$

the zinc(II)–imidazole (A) system, suggesting that the metal–ligand binding site in the ZnAH histamine complex is the imidazole nitrogen of histamine and thus the extra proton in this protonated complex must reside on the primary amino-group of the ligand. The same types of co-ordination sites have also been suggested [18] for the CuAH histamine complex.

A tetrahedral geometry involving imidazole and primary amino-nitrogens of the two histamine ligands is expected for the ZnA_2 histamine complex. The lesser difference between log K^{Zn}_{ZnA} and log $K^{ZnA}_{ZnA_2}$ values in Table 2 for the zinc(II)–histamine (A) system suggests that formation of both the ZnA and ZnA_2 complexes is favoured by the stability-increasing effect of the π-electron system of the imidazole group, as suggested by Gergely and Sovago.[16] It was concluded from single-crystal X-ray studies [21,22] that the complex bis(L-histidinato)zinc(II) consists of two molecules of L-histidine chelated to a zinc atom through the imidazole and primary amino-nitrogens forming stable six-membered

TABLE 1

Stability constants for the parent binary complexes of imidazole, histamine, and L-histidine with zinc(II) at 37 °C and I = 0.15 mol dm^{-3} ($Na[ClO_4]$). Standard deviations are given in parentheses

(*a*) Zinc(II)–imidazole (A) system

log β_{HA} * (pK_{NH^+})	log β_{ZnA} (log K^{Zn}_{ZnA})	log β_{ZnA_2}	log β_{ZnA_3}	log β_{ZnA_4}	log $K^{ZnA}_{ZnA_2}$	log $K^{ZnA_2}_{ZnA_3}$	log $K^{ZnA_3}_{ZnA_4}$
6.55(2)	2.55(9)	4.98(8)	7.40(10)	9.59(11)	2.43	2.42	2.19

(*b*) Zinc(II)–histamine and –L-histidine (A) systems

Ligands (A)	log β_{HA} *	log β_{H_2A} *	log β_{H_3A} *	log β_{ZnAH}	log β_{ZnA}	log $\beta_{ZnA_2H_2}$	log β_{ZnA_2H}	log β_{ZnA_2}
Histamine	9.39(8)	15.34(1)	—	11.91(5)	5.39(3)	—	—	10.45(4)
L-Histidine	8.96(3)	14.96(5)	17.37(9)	—	6.41(2)	22.80(12)	17.47(9)	11.74(2)

Ligands (A)	$pK_{NH_2^+}$	pK_{NH^+}	pK_{COOH}	pK^{H}_{ZnAH}	log K^{Zn}_{ZnA}	$pK^{H}_{ZnA_2H_2}$	$pK^{H}_{ZnA_2H}$	log $K^{ZnA}_{ZnA_2}$
Histamine	9.39	5.95	—	6.52	5.39	—	—	5.06
L-Histidine	8.96	6.00	2.41	—	6.41	5.33	5.73	5.33

* Value from ref. 18.

rings, the four nitrogens forming a tetrahedral array about zinc(II). The two carboxy oxygens approach the metal closely enough to be considered as loosely co-ordinated. The same structural characteristics can be assigned for the ZnA_2 L-histidine complex in solution also by considering the stability constant data in Table 1, *i.e.*, the log $K^{ZnA}_{ZnA_2}$ values for the L-histidine and histamine complexes do not differ much, suggesting that the tetrahedral sites of zinc(II) in both the systems are filled up by the same type of donor groups. The slightly higher log $K^{ZnA}_{ZnA_2}$ value for the ZnA_2 L-histidine complex compared to that for the histamine complex (Table 1) may be attributed to the carboxyl co-ordination in the former. The difference in log $K^{ZnA}_{ZnA_2}$ values for the L-histidine and histamine complexes would have been higher, if in the ZnA_2 L-histidine complex, one L-histidine was bound in a histamine-like and the other in a glycine-like fashion, as reported [18] for the CuA_2 L-histidine complex.

As described earlier, the extra proton in the ZnAH histamine complex resides on the primary amino-group of histamine. Since the $pK_{NH_3^+}$ and pK_{NH^+} values in histidine complex one of the two L-histidine ligands binds to the metal through its imidazole group with its primary amino-group being protonated and carboxy group remaining free and the other L-histidine molecule co-ordinates in a tridentate manner; thus altogether this complex species would have a tetrahedral geometry. This structure would additionally be favoured by the same type of electrostatic interaction described in the case of the ZnA_2H_2 L-histidine complex.

The concentration distribution diagrams in terms of percent bound zinc(II) as a function of pH were obtained for solutions of various metal : ligand ratios in the zinc(II)–imidazole, –histamine, and –L-histidine systems. As expected, the 1 : 1 complexes were found to predominate in 1 : 1 solutions, while the 1 : 2 complexes were more favoured in solutions containing excess of ligands. The diagrams obtained for a 1 : 4 solution in the zinc(II)–imidazole system and 1 : 2 solutions in zinc(II)–histamine and –L-histidine systems are given in Figure 1.

Mixed-ligand Systems of Zinc(II) involving Imidazole, Histamine, and L-Histidine as Ligands.—Three mixed-ligand systems, namely (i) zinc(II)–imidazole (A)–

TABLE 2

Stability constants for the mixed-ligand systems of zinc(II) involving imidazole, histamine, and L-histidine as ligands at 37 °C and I = 0.15 mol dm^{-3} ($Na[ClO_4]$). Standard deviations are given in parentheses

(*a*) Zinc(II)–imidazole (A)–secondary ligand (B) systems

Secondary ligands (B)	log β_{ZnAB}	log β_{ZnA_2B}	log K^{ZnA}_{ZnAB}	log K^{ZnB}_{ZnAB}	Δlog K_{ZnAB}	log X_{ZnAB}	log β_{ZnAB} (calc.)
Histamine	8.25(3)	10.35(17)	5.70	2.86	0.31	1.07	7.92
L-Histidine	9.50(6)	—	6.95	3.09	0.54	2.28	8.57

Secondary ligands (B)	Δlog β_{ZnAB}	log $K^{ZnAB}_{ZnA_2B}$	log $K^{ZnA_2}_{ZnA_2B}$	log $K^{ZnB}_{ZnA_2B}$	Δlog K_{ZnA_2B}	log X_{ZnA_2B}	log β_{ZnA_2B} (calc.)	Δlog β_{ZnA_2B}
Histamine	0.33	2.10	5.37	4.96	−0.02	0.64	10.32	0.03
L-Histidine	0.93	—	—	—	—	—	—	—

(*b*) Zinc(II)–histamine (A)–L-histidine (B) system

log β_{ZnAB}	log β_{ZnABH}	log β_{ZnABH_2}	log K^{ZnA}_{ZnAB}	log K^{ZnB}_{ZnAB}	Δlog K_{ZnAB}	log X_{ZnAB}	log β_{ZnAB} (calc.)	Δlog β_{ZnAB}
11.78(5)	18.58(5)	24.23(13)	6.39	5.37	−0.02	1.37	11.40	0.38

pK^H_{ZnABH}	Δlog K_{ZnABH}	log β_{ZnABH} (calc.)	Δlog β_{ZnABH}	$pK^H_{ZnABH_2}$	log β_{ZnABH_2} (calc.)	Δlog β_{ZnABH_2}
6.80	0.26	18.08	0.50	5.65	24.11	0.12

Table 1 for both histamine and L-histidine are comparable, it may be concluded that in the ZnA_2H_2 L-histidine complex also, the extra protons are attached to the primary amino-groups of the two L-histidines. Now the question arises regarding the metal–ligand binding in the ZnA_2H_2 L-histidine complex. The value of log P' [equation (2)] of 4.88 is comparable to the log β_{ZnA_2} value of 4.98 in the zinc(II)–imidazole (A) system,

$$\log P' = \log \beta_{Zn(AH)_2} - 2 \log \beta_{HA} \qquad (2)$$

demonstrating that only the imidazole nitrogens of two L-histidine ligands are involved in the co-ordination with the metal in this complex species and the carboxy groups of both the ligands remain free. This structure would be more favourable because of the electrostatic interaction between the protonated primary NH_3^+ group and the COO^- group in the L-histidine ligands. Thus, by considering these structural aspects of the ZnA_2H_2 L-histidine complex, it may be expected that in the ZnA_2H L-histamine (B), (ii) zinc(II)–imidazole (A)–L-histidine (B), and (iii) zinc(II)–histamine (A)–L-histidine (B) are discussed in this section.

The system (i) showed the presence of two mixed complexes, ZnAB and ZnA_2B, in addition to the binary complexes (HA, ZnA, ZnA_2, ZnA_3, ZnA_4, HB, H_2B, ZnBH, ZnB, and ZnB_2). The detection of ZnA_2B mixed species in this system demonstrates that zinc(II) prefers to be four-co-ordinate. In the system (ii), only the ZnAB type of mixed species was detected in addition to the binary complexes (HA, ZnA, ZnA_2, ZnA_3, ZnA_4, HB, H_2B, H_3B, ZnB, ZnB_2H_2, ZnB_2H, and ZnB_2). The values of log K^{ZnB}_{ZnAB} in Table 2 for both the systems (i) and (ii) are comparable to the value of log K^{Zn}_{ZnA} in the zinc(II)–imidazole (A) binary system (Table 1), suggesting that imidazole (A) is monodentate in these ZnAB mixed complexes. Also, comparison of the values of log K^{ZnA}_{ZnAB} in Table 2 for the systems (i) and (ii) with the log K^{Zn}_{ZnB} values in the zinc(II) binary systems of hista-

mine and L-histidine (Table 1) demonstrate that histamine and L-histidine (B) are bidentate and tridentate in their respective ZnAB mixed complexes. Thus, the non-detection of the ZnA_2B mixed species in system (ii) may be attributed to the fact that zinc(II) usually prefers to have a tetrahedral geometry and in the system under consideration even in the ZnAB mixed species, all the four positions in the tetrahedral plane would be occupied by the ligand molecules; *i.e.* three positions would be filled up by the tridentate binding of L-histidine (B) and the fourth position would be completed by the imidazole (A) molecule.

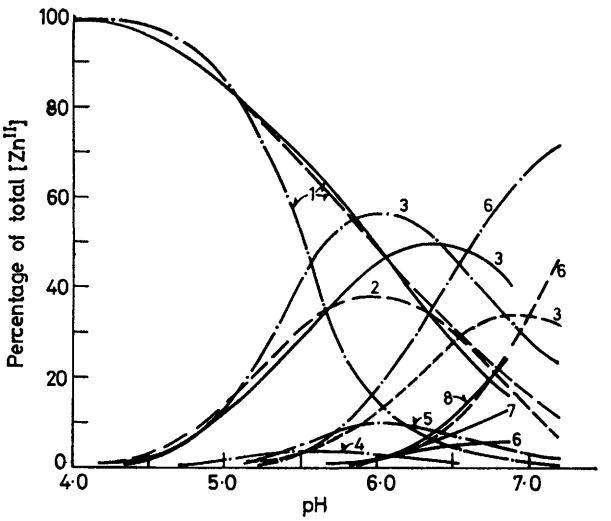

FIGURE 1 Species distribution for the binary systems of zinc(II) with imidazole (——), pH range 4.00—6.87, histamine (— — —), pH range 4.00—7.20, and L-histidine (— · —), pH range 4.00—7.20 (A), respectively at a metal–ligand ratio of 1 : 4, 1 : 2, and 1 : 2: (1) Unbound zinc(II), (2) ZnAH, (3) ZnA, (4) ZnA_2H_2, (5) ZnA_2H, (6) ZnA_2, (7) ZnA_3, and (8) ZnA_4

The parameters generally used for indicating the stabilization of the mixed complexes with respect to the binary ones,[2] namely, (*a*) $\Delta \log K$, the difference between the stabilities of the binary and mixed complexes, (*b*) $\log X$, the disproportionation constant, and (*c*) $\Delta \log \beta$, the stabilization constant which results from the difference of the stability constant measured for the mixed complex and that calculated from statistical grounds, are computed for the various mixed complexes detected in this study. On statistical grounds,[2] considerably less negative $\Delta \log K$ and more positive $\log X$ and also $\Delta \log \beta$ values indicate the marked stabilities of the mixed complexes. The values of $\Delta \log K$ [equations (3) and (4)], $\log X$ [equations (5) and (6)], and $\Delta \log \beta$ for the ZnAB

$$ZnA + ZnB \rightleftharpoons ZnAB + Zn \quad (3)$$

$$\Delta \log K_{ZnAB} = \log \beta_{ZnAB} - (\log \beta_{ZnA} + \log \beta_{ZnB}) \quad (4)$$

$$ZnA_2 + ZnB_2 \rightleftharpoons 2\,ZnAB;\ X_{ZnAB} = \frac{[ZnAB]^2}{[ZnA_2][ZnB_2]} \quad (5)$$

$$\log X_{ZnAB} = 2 \log \beta_{ZnAB} - (\log \beta_{ZnA_2} + \log \beta_{ZnB_2}) \quad (6)$$

complexes in the systems (i) and (ii) (Table 2) follow this trend. This suggests the marked stabilities of the CuAB complexes in both the systems (i) and (ii) which may be due to the fact that the π-electron system of the imidazole group in both the primary (A) and secondary (B) ligands favoured the mixed-ligand complex formation. Comparison of the $\log K^{ZnAB}_{ZnA_2B}$ value of 2.10 in the system (i) with the $\log K^{ZnA}_{ZnA_4}$ value of 2.19 in the zinc(II)–imidazole (A) system suggests that formation of ZnA_2B is less favoured. The same argument becomes more clear if it is noted that $\Delta \log K$, $\log X$ [equations (7)—(10)], and $\Delta \log \beta$, the stabilization constant values in Table 2 for the ZnA_2B mixed species in the system (i), do not deviate much from the statistically expected values. Comparison of the $\Delta \log K$ values in Table 2 for the ZnAB and ZnA_2B mixed complexes in system (i) suggests that the former species is more stable than the latter, which is in agreement with the general observation that in the mixed-ligand systems the unsaturated complex species (*e.g.* ZnAB) would be more stable than the saturated mixed species of the type ZnA_2B.

$$ZnA_2 + ZnB \rightleftharpoons ZnA_2B + Zn \quad (7)$$

$$\Delta \log K_{ZnA_2B} = \log \beta_{ZnA_2B} - (\log \beta_{ZnA_2} + \log \beta_{ZnB}) \quad (8)$$

$$ZnA_4 + ZnB_2 \rightleftharpoons 2\,ZnA_2B;\ X_{ZnA_2B} = \frac{[ZnA_2B]^2}{[ZnA_4][ZnB_2]} \quad (9)$$

$$\log X_{ZnA_2B} = 2 \log \beta_{ZnA_2B} - (\log \beta_{ZnA_4} + \log \beta_{ZnB_2}) \quad (10)$$

The mixed-ligand system (iii) under study showed the presence of three mixed species (ZnAB, ZnABH, and $ZnABH_2$) in addition to the binary complexes (HA, H_2A, ZnAH, ZnA, ZnA_2, HB, H_2B, H_3B, ZnB, ZnB_2H_2, ZnB_2H, and ZnB_2). It may be noted that Sovago *et al.*,[14] who also studied this system, found only one mixed species, ZnAB. However, the $\log \beta_{ZnAB}$ value of 11.48 reported by them is in good agreement with the value of 11.78 in the present investigation (Table 2) after making allowances for the changes in experimental conditions. Comparison of $\Delta \log K$, $\log X$ [equations (3)—(6)], and $\Delta \log \beta$, the stabilisation constant values in Table 2 for the ZnAB mixed species in the system (iii), with those values expected on statistical grounds [2] clearly suggests its marked stability. Again, the $\log K^{ZnB}_{ZnAB}$ and $\log K^{ZnA}_{ZnAB}$ values in Table 2 for this system are comparable to the stability constants in Table 1 for the zinc(II)–histamine and –L-histidine binary systems, indicating that histamine and L-histidine are respectively bidentate and tridentate in their ZnAB mixed species.

It appears that in the monoprotonated mixed species, ZnABH in the system (iii), the extra proton resides with the histamine (A) ligand, possibly on its primary amino-group (as is the case with the ZnAH histamine complex) because its pK^{H}_{ZnABH} value of 6.80 is comparable with the pK^{H}_{ZnAH} value of 6.52 in the zinc(II)–histamine (A) binary system. However, the $pK^{H}_{ZnABH_2}$ value of 5.65 for the diprotonated mixed species in this system compares favourably with the $pK^{H}_{ZnB_2H}$ value of 5.73 for the ZnB_2H L-histidine complex. Thus, in the $ZnABH_2$

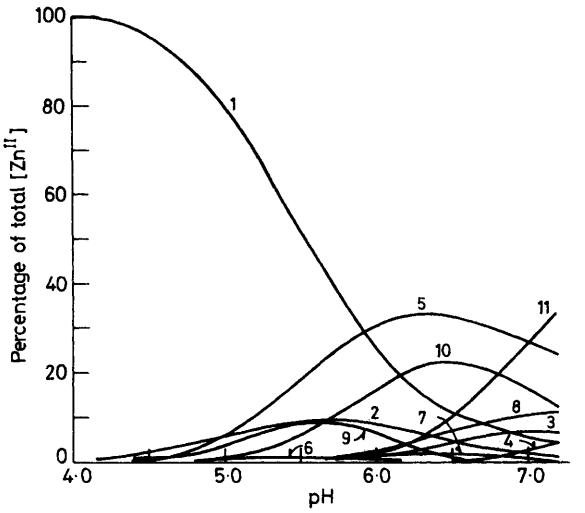

FIGURE 2 Species distribution for the zinc(II)–histamine (A)–L-histidine (B) mixed-ligand system at a metal : A : B ratio of 1 : 1 : 1 (pH range 4.00—7.20). (1) Unbound zinc(II), (2) ZnAH, (3) ZnA, (4) ZnA_2, (5) ZnB, (6) ZnB_2H_2, (7) ZnB_2H, (8) ZnB_2, (9) $ZnABH_2$, (10) ZnABH, and (11) ZnAB

mixed species in the system (iii), two protons would reside with the primary amino-groups of histamine (A) and L-histidine (B) ligands. In order to define the parameters $\Delta \log K$ and $\log X$, in the case of ZnABH and $ZnABH_2$ mixed complexes, the exact ligand being protonated must be taken into consideration. From the discussions given above, it appears that these parameters may be defined by expressions (11)—(18). The values of $\log X_{ZnABH}$, $\Delta \log K_{ZnABH_2}$, and $\log X_{ZnABH_2}$ could not be calculated because the stability constant data for

$$ZnAH + ZnB \rightleftharpoons ZnABH + Zn \quad (11)$$

$$\Delta \log K_{ZnABH} = \log \beta_{ZnABH} - (\log \beta_{ZnAH} + \log \beta_{ZnB}) \quad (12)$$

$$ZnA_2H_2 + ZnB_2 \rightleftharpoons 2\,ZnABH;$$

$$X_{ZnABH} = \frac{[ZnABH]^2}{[ZnA_2H_2][ZnB_2]} \quad (13)$$

$$\log X_{ZnABH} = 2 \log \beta_{ZnABH} - (\log \beta_{ZnA_2H_2} + \log \beta_{ZnB_2}) \quad (14)$$

$$ZnAH + ZnBH \rightleftharpoons ZnABH_2 + Zn \quad (15)$$

$$\Delta \log K_{ZnABH_2} = \log \beta_{ZnABH_2} - (\log \beta_{ZnAH} + \log \beta_{ZnBH}) \quad (16)$$

$$ZnA_2H_2 + ZnB_2H_2 \rightleftharpoons 2\,ZnABH_2;$$

$$X_{ZnABH_2} = \frac{[ZnABH_2]^2}{[ZnA_2H_2][ZnB_2H_2]} \quad (17)$$

$$\log X_{ZnABH_2} = 2 \log \beta_{ZnABH_2} - (\log \beta_{ZnA_2H_2} + \log \beta_{ZnB_2H_2}) \quad (18)$$

ZnA_2H_2 and ZnBH [equations (14), (16), and (18); Table 1] could not be obtained. $\Delta \log K$ and $\Delta \log \beta$, the stabilization constant values in Table 2 for the species ZnABH, clearly indicate its increased stability. Again, the stabilization constant $\Delta \log \beta_{ZnABH_2}$ in Table 2 is positive, suggesting the marked stability of $ZnABH_2$ mixed species compared to the protonated binary complexes of histamine (A) and L-histidine (B) with zinc(II).

The distribution of various binary and mixed-ligand complex species was obtained for all the three mixed-ligand systems in this study in terms of percent bound zinc(II) as a function of pH. Though increased stabilities compared to the statistical case were observed for most of the mixed-ligand complex species detected, in none of these systems did the maximum amount of the total zinc(II) found to be present in the form of mixed-ligand species [19, 41, and 34% respectively in the systems (i), (ii), and (iii) in ZnAB forms] exceed the statistically expected [2] 50% with regard to parent binary species. Of course, this is not surprising because usually the amount of mixed species with zinc never exceeds 50%, and more often it is nearer 30%. The concentration distribution diagram for the zinc(II)–histamine (A)–L-histidine (B) system is given in Figure 2; the diagrams for the other two systems were qualitatively similar.

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