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Mixed Aminocarboxylic Ligand Complexes

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The stabilities of some mixed proton complexes $AA'H_j$ (A = histidine (His), lysine (Lys), histamine (Hist); A' = bi-, tri- and tetra-carboxylic acids, aspartic acid and glutamic acid) have been determined by potentiometry. Different species in each system have been found with $j = 1, \ldots, (n + m) - 1$ (n and m are the maximum degrees of protonation for the two ligands). The stability of a mixed species is strictly dependent on the charges involved in the formation reactions, and a simple relationship $\log K = f(z_T)$ (where z_T is the sum of charges in the two ligands) is reported.

In the last ten years extensive research on the formation of weak species in aqueous solution and on the dependence of protonation and formation constants on the ionic medium has been performed in our laboratories.[1–6] From the study of the protonation constants of amines in various ionic media and at different ionic strengths, we reached the conclusion that the experimental data could be explained by the formation of weak complex species between the protonated amine and the anion of the ionic medium.[7–11] The scope of this research was subsequently broadened to include possible interactions of protonated polyamines with inorganic and organic polyanions.[12–16] These studies enabled us to prove that polyamines and polyanions form complexes which are highly dependent on the positive charges of the protonated amine and on the negative sites of the second ligand. In some cases, formation constant values are so high that it is no longer appropriate to speak of weak interactions, nor can these complexes be ignored in the speciation of fluids containing the previously mentioned classes of ligands. Little other published research exists in this field and what there is covers mainly the formation of cyclic polyamine complexes with bi- and tri-carboxylic acids and with the polyphosphates,[17–22] adenosine 5′-mono-, di- and tri-phosphates (AMP, ADP and ATP, respectively).

We now consider another class of ligands, amino acids, which, having both anionic and cationic characteristics, can form complexes both with polyanions[21] and protonated amines. The stabilities of carboxylate–amino acid, amino acid–amine or amino acid–amino acid complexes are probably fairly low on the basis of past experience, with the exception of some special cases (amino acids with more than one amino group which form complexes with highly charged polyanions), and may be very difficult to determine quantitatively because of the concomitant weak interactions of carboxylic groups with alkaline cations and of protonated amino groups with inorganic anions. This study deals both with interactions between amino acids having one or more *N*-donor sites and polyprotic carboxylic acids, and with those between amino acids themselves. In addition, in order to identify the influence of the carboxylic group in the His molecule, considered here as an *N*-ligand, we also studied complexes of Hist with carboxylic acids. The study was performed in aqueous solution at $T = 25\,^{\circ}C$, by means of the glass electrode potentiometric technique. We report here the results on the stabilities of mixed complex species formed between (*a*) protonated His with succinate (succ), citrate (cit), tricarballylate (1,2,3-propanetricarboxylate, tca), glutamate (Glu), and aspartate (Asp); (*b*) protonated Lys with Glu, and tca; (*c*) protonated Hist with succ, cit, tca, butanetetracarboxylate (btc), and Asp.

Experimental

Materials

Glutamic and aspartic acids, histidine and lysine hydrochlorides, as well as histamine and ethylenediamine hydrochlorides (Fluka BioChemika or *puriss.* p.a.) were used as supplied; their purity was checked by potentiometric titrations and always found to be $\geqslant 99.6\%$. Succinic (H_2succ), citric (H_3cit) and butanetetracarboxylic (H_4btc) acids, were also used as supplied; their purity was checked alkalimetrically and found to be $\geqslant 99.4\%$. Tricarballylic acid (H_3tca) was purified by recrystallization according to Perrin *et al.*[23] from the *purum* product; the final product collected had a purity $>99.4\%$. NaCl (Fluka *puriss.* p.a.) was vacuum-dried and the stock solution was prepared by weighing. HCl and NaOH solutions were prepared by diluting concentrated ampoules (Carlo Erba) and standardized against sodium carbonate and potassium biphthalate (Fluka *puriss.* p.a.), respectively, and were protected from atmospheric CO_2 by soda lime traps. All solutions were prepared with twice-distilled demineralized water and grade A glassware.

Apparatus

Free proton concentration measurements were performed by using a Metrohm mod. 654 potentiometer, coupled with an Orion combination glass electrode Ross type mod. 8102, and a motorized burette Metrohm mod. 665; both were connected to a PC for the titration control and automatic acquisition of data. The instrumental resolution was 0.1 mV and the reproducibility was ± 0.15 mV. Measurement cells were thermostatted at $T = 25 \pm 0.1\,^{\circ}C$, and purified N_2 was bubbled into the solutions in order to exclude atmospheric CO_2.

Procedure

The solutions under examination (25 cm^3), containing the pairs of ligands in variable concentrations (see Table 1; charges are omitted in all tables for the sake of simplicity), were potentiometrically titrated up to a pH of *ca.* 11 using standard NaOH; these contained no background salt in order to minimize the effects of interactions with Na^+ (from the titrant) and Cl^- (from the amine or amino acid

Table 1 Experimental details of potentiometric measurements, at 25 °C

A	A′	C_A[a]	$C_{A'}$[a]	$C_A/C_{A'}$	pH	I[a]	N_{tit}[b]	N_{exp}[c]
His	succ	0.010–0.080	0.010–0.080	0.125–8	1.6–8	0.09–0.20	9	942
	cit	0.005–0.050	0.005–0.050	0.22–10	1.6–9	0.08–0.20	9	683
	tca	0.005–0.050	0.004–0.050	0.11–12	1.6–8	0.03–0.19	10	942
	Asp	0.010–0.060	0.010–0.060	0.25–4	2–9	0.06–0.15	17	820
	Glu	0.010–0.080	0.010–0.080	0.125–8	1.6–9	0.03–0.16	8	766
Lys	tca	0.004–0.042	0.005–0.050	0.08–8	1.7–10.5	0.04–0.15	10	875
	Glu	0.008–0.067	0.011–0.082	0.10–6	1.6–10.5	0.04–0.19	8	683
Hist	succ	0.020–0.050	0.020–0.060	0.3–2.5	4–9.3	0.07–0.19	4	211
	cit	0.020–0.050	0.020–0.040	0.5–2.5	2.3–9.6	0.09–0.18	4	295
	tca	0.020–0.050	0.020–0.040	0.5–2.5	3–9.3	0.09–0.19	4	256
	btc	0.020–0.050	0.010–0.030	0.7–1.6	3–9.2	0.09–0.19	4	258
	Asp	0.020–0.050	0.020–0.060	0.3–2.5	4.5–9.3	0.06–0.15	4	136

[a] Analytical concentrations (C) and ionic strengths (I) in mol dm^{-3}. [b] N_{tit} is the number of titrations. [c] N_{exp} is the number of experimental points.

hydrochlorides). The standard potential value (E^0) of the electrode couple and j_a, the coefficient of the junction potential ($E_j = j_a[H]$), were calculated through the titration of HCl (5–20 mmol dm^{-3}) in the same ionic strength conditions as the solutions under examination (ionic strength adjusted with NaCl).

Calculations

Since in the study of weak complexes the constant ionic medium method cannot generally be used, it is necessary to have a relationship between the activity coefficients and the ionic strength. In research carried out in this laboratory it has been demonstrated that in the ionic strength range $0 \leqslant I/\text{mol dm}^{-3} \leqslant 1$, a Debye–Hückel type equation can be used:[3,6]

$$\log \beta = \log {}^{T}\beta - z^*\sqrt{I}/(2 + 3\sqrt{I}) + CI + DI^{3/2}$$

where β is the generic formation constant and ${}^{T}\beta$ is the formation constant at infinite dilution. The empirical parameters C and D can in turn be expressed by

$$C = c_0 p^* + c_1 z^*; \qquad D = dz^*$$

where

$$p^* = \sum \text{mol}_r - \sum \text{mol}_p$$

$$z^* = \sum (\text{charges})_r^2 - \sum (\text{charges})_p^2$$

(r and p represent reactants and products, respectively). In this work we used $c_0 = 0.1$, $c_1 = 0.23$ and $d = -0.1$. The dependence on ionic strength of E^0 and j_a was also taken into consideration.[6]

For both the determination of formation constants and speciation analysis of the systems under examination we used a number of PC programs summarized below:
(i) BSTAC:[6] This program, based on the non-linear least-squares method, refines the formation constants of complexes, from pH-metric measurements, and the analytical parameters, minimizing the $U = \sum (E_{exp} - E_{calc})^2$ function (E = emf). It takes into consideration the variations in ionic strength. (ii) STACO:[24] This is similar to the previous program in being designed to identify parameters to be refined and minimizing the $U = \sum (v_{exp} - v_{calc})^2$ function (v is titrant volume). (iii) ES4EC:[6] This program calculates concentrations of all species present in a multicomponent solution from the formation constants of the various complexes and the total concentrations of the different components of the system, even under variable ionic strength conditions.[7] (iv) ESAB:[25] This program was used for the standardization of the electrode couple and the calculation of the purity of reagents.

Results

Simple Systems

The protonation and the complex formation of carboxylic acids with Na^+, and the protonation and complexation with Na^+ and Cl^- of amines and amino acids have already been studied[1,4,5,7–9,26] and the relative constants are reported in Table 2. From the values of the Na^+ and Cl^- formation complex constants, it can clearly be concluded that to ignore these interactions would mean to fail to define the speciation model correctly, and therefore we included in all the calculations the formation of these species too.

Mixed Ligand–Ligand Complexes

Almost all the systems studied form $AA'H_j$ with $j = 1, \ldots, (m + n) - 1$ type species where m is the maximum degree of amine or amino acid protonation and n is the number of protons of the carboxylic acid. The speciation model for the various systems was obtained by using some simple rules. Several models were considered using the computer and the procedure for selecting the best one included the following points: (*a*) check for the absence of systematic errors; (*b*) choice of a suitable weighing scheme; (*c*) use of the F-test for the selection of the most suitable speciation model; (*d*) a check for the absence of systematic trends in the residuals, in order to avoid bias in the selection of species. By using this procedure it was found that species containing more than one molecule from one of the two ligands are not formed.

Mixed Complexes of His

Table 3 shows the overall and partial formation constants of the His-H-succ, -cit and -tca systems. For these systems respectively, four, five and five [general formula (His) $A'H_j$; $j_{max} = 4$, 5, 5, respectively] mixed species were identified. Closer examination of the data obtained shows that the partial formation constants keep within a fairly restricted range of values. For example, succ with proton and His forms four fairly stable complexes with log K ranging between 2.1 and 1.2; note that the logarithmic values of the partial stability constants increase until the $H_2His^{2+} + succ^{2-}$ reaction, and then decrease. Similar behaviour was observed in the other systems studied, for which the log K values ranged from a minimum of 1.2, for the reaction of the monoprotonated species, to a maximum of 2.5, for the reaction of the diprotonated species [$H_2His + A'$], then dropped again

Table 2 Protonation and formation constants (expressed as log β_{pqr}[a]) with Na^+ and Cl^-, of carboxylic acids, amines and amino acids considered in this work, at $I = 0$ mol dm^{-3} and $T = 25$ °C

pqr	succ[b]	cit[c]	tca[d]	btc[e]	Hist[f]	en[g]	Asp[h]	Glu[f]	Lys[e]	His[h]
001	5.64	6.418	6.491	7.18	9.81	9.91	10.01	9.98	10.82	9.30
002	9.85	11.169	11.406	13.01	15.74	16.77	13.89	14.37	19.89	15.29
003	—	14.301	15.089	17.54	—	—	15.81	16.56	21.66	16.87
004	—	—	—	20.92	—	—	—	—	—	—
100	0.85	1.54	1.398	1.82	—	—	0.42	0.35	−0.27	−0.5
101	5.79	7.29	7.308	8.67	—	—	9.73	9.7	—	—
102	—	11.27	11.558	13.94	—	—	—	—	—	—
103	—	—	—	17.76	—	—	—	—	—	—
200	—	2.34	1.981	3.45	—	—	—	—	—	—
201	—	7.01	6.959	9.31	—	—	—	—	—	—
300	—	—	—	3.30	—	—	—	—	—	—
011	—	—	—	—	9.4	9.8	—	—	10.52	—
012	—	—	—	—	15.95	17.43	—	—	20.14	15.22
013	—	—	—	—	—	—	15.34	16.1	22.58	17.28

[a] $\beta_{pqr} = [Na_pLCl_qH_r]/([Na]^p[L][Cl]^q[H]^r)$; L = ligand. [b] Ref. 1. [c] Ref. 26. [d] Ref. 5. [e] Ref. 4. [f] Unpublished data from these laboratories. [g] Ref. 7. [h] Ref. 8.

Table 3 Overall and partial formation constants of proton-His-succ, -cit and -tca complexes, at $T = 25$ °C and $I = 0$ mol dm^{-3}

	log $\beta \pm 3s$[a]		
overall reactions	A′ = succ	A′ = cit	A′ = tca
His + A′ + H → (His)A′H	11.08 ± 0.09	10.46 ± 0.15	10.50 ± 0.15
His + A′ + 2H → (His)A′H_2	17.43 ± 0.08	17.81 ± 0.12	17.80 ± 0.08
His + A′ + 3H → (His)A′H_3	22.54 ± 0.08	23.76 ± 0.13	23.80 ± 0.06
His + A′ + 4H → (His)A′H_4	26.35 ± 0.06	28.32 ± 0.13	28.53 ± 0.06
His + A′ + 5H → (His)A′H_5	—	31.33 ± 0.10	32.02 ± 0.06
	log $K \pm 3s$[a]		
partial reactions	A′ = succ	A′ = cit	A′ = tca
HHis + A′ → (His)A′H	1.8 ± 0.2	1.2 ± 0.2	1.2 ± 0.3
HHis + HA′ → (His)A′H_2	—	2.1 ± 0.2	2.0 ± 0.2
H_2His + A′ → (His)A′H_2	2.1 ± 0.1	2.5 ± 0.2	2.5 ± 0.1
H_2His + HA′ → (His)A′H_3	1.6 ± 0.1	2.0 ± 0.2	2.0 ± 0.1
H_2His + H_2A′ → (His)A′H_4	1.2 ± 0.1	1.9 ± 0.2	1.8 ± 0.1
H_2His + H_3A′ → (His)A′H_5	—	1.7 ± 0.2	1.6 ± 0.1

[a] s = standard deviation.

and stabilized at lower values for the formation of the penta-protonated species. This behaviour is correlated to the charge value of the reactants.

Table 4 shows the overall and partial formation constants of the His-H-Asp and -Glu systems. For both systems, five mixed species with general formula (His)A′H_j ($j_{max} = 5$), were found. As can be seen, the behaviour of the values of the formation constants is fairly consistent even considering different ligands (justified by the great similarity of Asp and Glu molecules); the species formed have stabilities ranging from a minimum value of log $K = 0.8$ for Asp, to a maximum value of log $K = 2.0$ for Glu.

The stabilities reported in Tables 3 and 4, in terms of formation percentages, mean that these mixed species are formed, at the appropriate pH value, in significant amounts when $C_r > 10$ mmol dm^{-3}. Fig. 1 shows the distribution of the mixed species in the His-H-Glu system *vs.* pH at $I = 0.1$ mol dm^{-3} and $T = 25$ °C. The main species is $[(His)(Glu)H_2]^-$; the other species however, as can be seen from the graph, are present in quite appreciable quantities. At pH = 7.5, more than 40% of His is bound to Glu in the reported experimental conditions.

Mixed Complexes of Lys

Table 5 shows the overall and partial formation constants of the Lys–tca and Lys–Glu systems. For these we have found five proton mixed species having general formula AA′H_j, with $j_{max} = 5$. Here too, with the exception of the (Lys)(Glu)H_4 species, average constant values are fairly high, indicating the formation of quite stable species. Marked similarity is to be found between the Lys–tca system and those

Table 4 Overall and partial formation constants of proton-His-Asp and -Glu complexes, at $T = 25$ °C and $I = 0$ mol dm^{-3}

	log $\beta \pm 3s$[a]	
overall reactions	A′ = Asp	A′ = Glu
His + A′ + H → (His)A′H	11.39 ± 0.15	11.58 ± 0.12
His + A′ + 2H → (His)A′H_2	20.42 ± 0.12	20.72 ± 0.12
His + A′ + 3H → (His)A′H_3	26.58 ± 0.10	27.00 ± 0.10
His + A′ + 4H → (His)A′H_4	30.01 ± 0.12	31.02 ± 0.09
His + A′ + 5H → (His)A′H_5	31.88 ± 0.15	33.19 ± 0.09
	log $K \pm 3s$[a]	
partial reactions	A′ = Asp	A′ = Glu
His + HA′ → (His)A′H	1.4 ± 0.2	1.6 ± 0.2
HHis + HA′ → (His)A′H_2	1.1 ± 0.2	1.4 ± 0.2
H_2His + HA′ → (His)A′H_3	1.3 ± 0.2	1.7 ± 0.2
H_2His + H_2A′ → (His)A′H_4	0.8 ± 0.2	1.4 ± 0.2
H_2His + H_3A′ → (His)A′H_5	0.8 ± 0.2	1.3 ± 0.2
H_3His + H_2A′ → (His)A′H_5	1.1 ± 0.2	2.0 ± 0.2

[a] s = standard deviation.

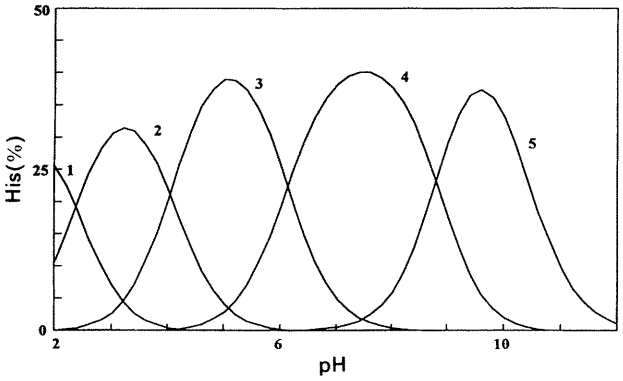

Fig. 1 Speciation diagram *vs.* pH in the system H^+-His-Glu at $I = 0.1$ mol dm^{-3} and $T = 25$ °C. Analytical concentrations: $C^0_{His} = C^0_{Glu} = 0.05$ mol dm^{-3}; $C^0_{Na} = C^0_{Cl} = 0.05$ mol dm^{-3}. Species: (1) $[His(Glu)H_5]^{2+}$, (2) $[His(Glu)H_4]^+$, (3) $[His(Glu)H_3]^0$, (4) $[His(Glu)H_2]^-$, (5) $[His(Glu)H]^{2-}$.

described in Table 3, in that maximum stability is always achieved as a result of the reaction between the biprotonated amino acid and the tca anion, with log $K = 2.2$. The log K values for the Lys–Glu system among which there is little variation, also imitate the behaviour already described for other similar systems (Table 4). Other mixed proton complexes of Lys have been already studied[16] and their stability behaviour is very similar to that of species reported here.

Fig. 2 shows the distribution of mixed species in the Lys-H-tca system *vs.* pH; note that the main species is $[(Lys)(tca)H_2]^{2-}$, which predominates in the pH range 6–10. Formation percentages, in the reported experimental conditions, are quite high, *i.e.* >25%, with respect to Lys.

Table 5 Overall and partial formation constants of proton-Lys-tca and -Glu complexes, at $T = 25$ °C and $I = 0$ mol dm^{-3}

	log $\beta \pm 3s^a$	
overall reactions	A′ = tca	A′ = Glu
Lys + A′ + H → (Lys)A′H	11.5 ± 0.3	11.70 ± 0.30
Lys + A′ + 2H → (Lys)A′H_2	22.09 ± 0.06	21.70 ± 0.20
Lys + A′ + 3H → (Lys)A′H_3	28.02 ± 0.06	30.87 ± 0.05
Lys + A′ + 4H → (Lys)A′H_4	32.75 ± 0.15	34.40 ± 0.10
Lys + A′ + 5H → (Lys)A′H_5	35.9 ± 0.4	37.20 ± 0.30
	log $K \pm 3s^a$	
partial reactions	A′ = tca	A′ = Glu
HLys + A′ → (Lys)A′H	0.7 ± 0.4	0.9 ± 0.4
HLys + HA′ → (Lys)A′H_2	—	0.9 ± 0.3
H_2Lys + A′ → (Lys)A′H_2	2.2 ± 0.1	—
H_2Lys + HA′ → (Lys)A′H_3	1.6 ± 0.1	1.0 ± 0.2
H_2Lys + H_2A′ → (Lys)A′H_4	1.4 ± 0.2	0.2 ± 0.2
H_2Lys + H_3A′ → (Lys)A′H_5	0.9 ± 0.4	0.8 ± 0.4

[a] s = standard deviation.

Mixed Complexes of Hist

Table 6 shows the overall and partial formation constants relative to systems Hist-H-succ, -cit, -tca, -btc and -Asp. Respectively j_{max} ($j_{max} = 2$, 4, 3, 4, 3) mixed species with general formula (Hist)A′H_j are formed. In this case, when A′ is the anion of a carboxylic acid, we observe the behaviour previously seen in the partial formation constant values: an initial increase in values followed by a quite considerable drop and a maximum log K value for the H_2 (Hist) + A′ reaction; in the case of the Hist-H-cit system, for example, the range of values goes from log $K = 2.1$ to a maximum of log $K = 3.2$ and then drops to a minimum of log $K = 0.8$. Table 6 also shows previously determined values for systems en-H-mal, -cit, -tca and -btc (en, ethylenediamine; mal, malonate). The trend followed by mixed complexes of ethylenediamine

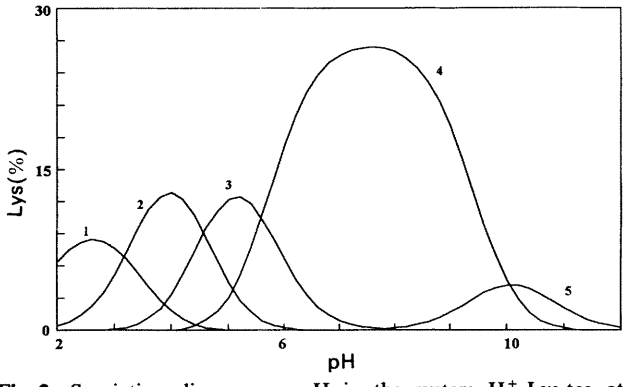

Fig. 2 Speciation diagram *vs.* pH in the system H^+-Lys-tca at $I = 0.1$ mol dm^{-3} and $T = 25$ °C. Analytical concentrations: $C^0_{Lys} = 0.03$ mol dm^{-3}; $C^0_{tca} = 0.02$ mol dm^{-3}; $C^0_{Na} = C^0_{Cl} = 0.05$ mol dm^{-3}. Species: (1) $[Lys(tca)H_5]^+$, (2) $[Lys(tca)H_4]^0$, (3) $[Lys(tca)H_3]^-$, (4) $[Lys(tca)H_2]^{2-}$, (5) $[Lys(tca)H]^{3-}$.

Table 6 Overall and partial formation constants of proton-Hist-succ, -cit, -tca, -btc and -Asp complexes, at $T = 25$ °C and $I = 0$ mol dm^{-3}

	log $\beta \pm 3s^a$				
overall reactions	A′ = succ	A′ = cit	A′ = tca	A′ = btc	A′ = Asp
Hist + A′ + H → (Hist)A′H	11.17 ± 0.05	11.89 ± 0.10	11.62 ± 0.05	12.25 ± 0.05	12.10 ± 0.15
Hist + A′ + 2H → (Hist)A′ + H_2	17.65 ± 0.03	18.93 ± 0.05	18.70 ± 0.03	19.74 ± 0.03	21.25 ± 0.15
Hist + A′ + 3H → (Hist)A′H_3	—	24.02 ± 0.05	23.90 ± 0.04	25.72 ± 0.03	26.80 ± 0.40
Hist + A′ + 4H → (Hist)A′H_4	—	27.66 ± 0.15	—	30.32 ± 0.15	—
	log $K \pm 3s^a$				
partial reactions	A′ = succ	A′ = cit	A′ = tca	A′ = btc	A′ = Asp
Hist + HA′ → (Hist)A′H	—	—	—	—	2.1 ± 0.3
H(Hist) + A′ → (Hist)A′H	1.4 ± 0.1 0.9[b]	2.1 ± 0.2 1.5[c]	1.8 ± 0.1 1.8[d]	2.4 ± 0.1 2.2[e]	—
H(Hist) + HA′ → (Hist)A′H_2	—	—	—	—	1.4 ± 0.2
H_2(Hist) + A′ → (Hist)A′H_2	1.9 ± 0.1	3.2 ± 0.1	3.0 ± 0.1	4.0 ± 0.1	—
H_2(Hist) + HA′ → (Hist)A′H_3	—	1.9 ± 0.2	1.7 ± 0.1	2.8 ± 0.1	1.1 ± 0.4
H_2(Hist) + H_2A′ → (Hist)A′H_4	—	0.8 ± 0.2	—	1.6 ± 0.2	—

[a] s = standard deviation. [b] mal-en.[12] [c] cit-en.[14] [d] tca-en.[14] [e] btc-en.[14]

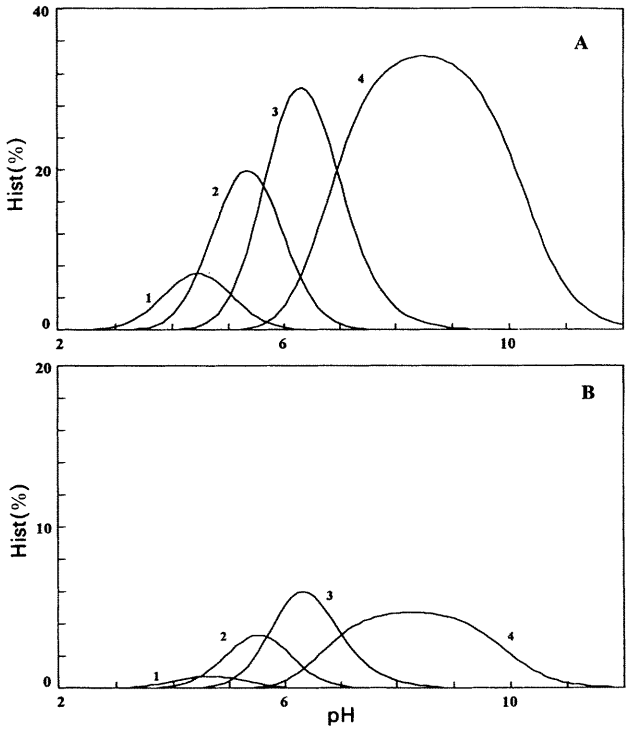

Fig. 3 Speciation diagram *vs.* pH in the system H^+-Hist-btc at $I = 0.1$ mol dm^{-3} and $T = 25\,°C$. Analytical concentrations: A, $C^0_{Hist} = C^0_{btc} = 0.03$ mol dm^{-3}; $C^0_{Na} = C^0_{Cl} = 0.05$ mol dm^{-3}; B, $C^0_{Hist} = C^0_{btc} = 0.002$ mol dm^{-3}; $C^0_{Na} = C^0_{Cl} = 0.05$ mol dm^{-3}. Species: (1) $[(Hist)(btc)H_4]^0$, (2) $[(Hist)(btc)H_3]^-$, (3) $[(Hist)(btc)H_2]^{2-}$, (4) $[(Hist)(btc)H]^{3-}$.

with carboxylic ligands is very similar to that followed by analogous Hist complexes. Fig. 3 shows the distribution of mixed species in the Hist-H-btc system *vs.* pH; the predominant species is $[(Hist)(btc)H]^{3-}$. In this case formation percentages are high even at low reactant concentrations (Fig. 3B), *i.e.* btc-H-Hist species are significant in less than mmol amounts.

The constants relating to the Hist-H-Asp system are, predictably, significantly lower than those of the corresponding systems with A′ = carboxylate because of the presence of the aminic group in Asp.

Discussion

Although the results in Tables 3–6 show that the formation of mixed complexes between the ligands considered is significant, the species formed show quite low stability (except Hist mixed species). In general, excepting Hist complexes, the logarithm of the equilibrium constant of partial reactions is lower than two. Two types of characteristic behaviour can be discerned from these data. The first concerns the obvious observation that an increase in the charges involved in the two ligands increases the stability of the species. The second is to do with the fact that the presence of different types of charge in the same ligand weakens the complex of which it is part. For example, the homologous series of His and Hist display differing stabilities in favour of Hist. Trends in stability in relation to the different resident charges can be rationalized by the use of simple equations. For example, in this laboratory it has already been found that ethylenediamine complexes with a series of carboxylic acids[14] display an equilibrium constant for species (en)A′H, (en)A′H_2, and (en)A′H_3 which can be expressed as a function of the charges by the following equation:

$$\log K = \log K_\infty + a/z_{res} + 4.3/z^2_{res}$$

where z_{res} is the residual charge of the carboxylic ligand. The empirical $\log K_\infty$ parameter may be seen as a limit for a hypothetical infinite number of carboxylic groups. The same type of analysis was carried out for Hist. For the reaction

$$H_j(Hist)^{j+} + A'^{z-} = (Hist)A'H_j^{(j-z)+}$$

we found $\log K_\infty = 4.0$, 4.5 ($j = 1$); 6.4, 6.1 ($j = 2$); and $a = -8.4$, -9.1 ($j = 1$); -10.0, -9.0 ($j = 2$) (the first and second values refer to en and Hist, respectively). Both $\log K_\infty$ and a parameters are quite close for en and Hist. From all the stability data contained in this study we may derive another empirical relationship between the partial stability constant of the ligand–ligand complex and the reduced effective total charge

$$z_T = |z(A)| + |z(A')| - |z_a|$$

where z_a represents the remaining charges which do not take part in the formation reaction. For example, for reaction $H_2His^+ + succ^{2-} = (His)(succ)H^-$, $z_T = 1 + 2 - 1 = 2$ ($z_a = 1$ is the Hist carboxylic charge). The relationship obtained is:

$$\log K = 1.22 + 0.226 z_T$$

These empirical expressions can be usefully employed for systems for which the stability of the complexes formed has not been experimentally determined. Finally, something must be said as to the importance of these species in natural systems. While these interactions are altogether unlikely to be of real interest in the speciation of natural fluids, it is certainly very important to take them into consideration from the point of view of intracellular concentrations and intramolecular interactions in macromolecules containing series of functional groups identical or similar to those considered in this study. Furthermore, in the light of the present results, we think that the stability of several metal–mixed-ligand complexes[27] must be recalculated, by considering also the formation of proton–mixed-ligand complexes.

The main conclusions can be summarized as follows:

(*a*) The binding of amino acids by carboxylic ligands leads to the formation of weak complexes with significant stability.

(*b*) The stability of these complexes depends mainly on the charges of the two ligands.

(*c*) Some empirical stability–charge relationships make it possible to predict $\log K$ values for low molecular weight ligand–ligand complexes.

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