Potentiometric and Spectroscopic Study of Equilibria in the Aqueous Ternary System Copper^{II}–L-Histidine–HA (HA = aminomethane- or 2-aminobenzene-sulfonic acid)

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Complexation in the ternary system Cu^{II}–L-histidine–HA [HA = aminomethanesulfonic acid (Hamsa) or 2-aminobenzenesulfonic acid (Habsa)] was investigated in aqueous 0.15 mol dm^{-3} NaCl solution at 25 °C by potentiometric and spectroscopic techniques. The results show, over the pH range 4–8 and Cu^{II}–L-histidine–HA ratios of 1:2–8:4–12, the presence of the species $[Cu(His)_2]$, $[Cu(HHis)(His)]^+$ and [Cu(His)A] [His = L-histidinate(1−)]. The log stability constants of the latter complexes were 15.85 (A = amsa) and 17.01 (A = absa). Neither ternary species is present at neutral pH, however, they are significant at lower pHs; this point is discussed in relation to rheumatoid arthritis therapy.

The elevation of the concentrations of low molecular weight copper species in the plasma and synovial fluids of rheumatoid arthritis patients is well documented.[1,2] This has led to a number of studies which indicate some involvement of copper in this disease; in particular, a number of Cu^{II} chelates have been shown to possess anti-inflammatory activity.[3,4] A reasonable conclusion from these studies is that the metal ions are unable to reach the ribosomes and so fail to become incorporated into relevant enzyme systems. This is indicative of a defective Cu transport mechanism operating either in the cytosol or the plasma compartments. This defect could be attributed to a copper 'carrier' being absent within the plasma with the result that the metal ions are unable to juxtapose the plasma membrane as an initial step into entering the cytosol. In this context there would appear to be a requirement for a ligand which can complex with copper and enhance the transport of the metal into the cytosol.

Histidine is a major ligand for copper in the low molecular weight fraction of blood plasma, computer simulations indicating that *ca.* 90% of the available Cu^{II} is complexed to this amino acid in binary and ternary forms.[5] For a ligand to be of potential application in the treatment of rheumatoid arthritis it must be able to either compete with histidine for copper in plasma or form ternary complexes of the type Cu–histidine–ligand.[6] If administered as a pre-formed Cu chelate[3] then, for the preferable oral administration, the chelate must be stable towards the low pH region of the digestive system. In this respect sulfonic groups are preferable to carboxylic groups (assuming also the need to neutralize the positive charge on the Cu ion).

It is generally accepted that the sulfonic group is a poor ligand for metal ions, attributable to its poor basicity. However, its co-ordination ability could be enhanced if incorporated into a chelating ligand. With the above requirements in mind we now report potentiometric and spectroscopic measurements on the aqueous ternary system Cu–L-histidine–HA where HA = aminomethanesulfonic acid (Hamsa) or 2-aminobenzenesulfonic acid (Habsa), in order to determine the co-ordinating properties of the sulfonic ligands and hence their potential as chelating anti-inflammatory compounds. No attempt was made to determine the binary CuA_n equilibrium constants as it was considered unlikely that such complexes would form under physiological conditions.

Results and Discussion

Analysis of Potentiometric Data.—The steps involved in determining the species present and their equilibrium constants using the suite of programs PLOT3, GUESS3 and BETACALC3 were as outlined previously.[7–9] The 'goodness of fit' criterion is based on achieving a minimum residual factor R, equation (1), where $(C_{expt} - C_{calc})$ represents the

$$R = \Sigma (C_{expt} - C_{calc})^2 \quad (1)$$

difference between the calculated and experimental concentration values summed over n titration points (typically $n \simeq 100$). The various equilibria are represented by the general expressions (2) and (3).

$$pM + qH + rA + sB = M_pH_qA_rB_s \quad (2)$$

$$\beta_{pqrs} = [M_pH_qA_rB_s]/[M]^p[H]^q[A]^r[B]^s \quad (3)$$

Ligand Protonation Constants.—The two protonation constants, for the ligands amsa and absa are given in Table 1. In both cases the second equilibrium constant β_{0210}, corresponding to protonation of the sulfonic group, was the more difficult to determine accurately because of the non-linearity of the electrode response in the low − log [H] region. This non-linearity is reflected in the large standard deviation observed for this constant. For the related compound 2-aminoethanesulfonic acid (taurine)[10] the values in the literature for the related K_a constant ($= \beta_{0210}/\beta_{0110}$) vary from −1.05 to +1.05, although under different solution conditions. However, for the same range of conditions the agreement of β_{0110} values is

Table 1 Stability constants log β_{pqrs} of the species $M_pH_qA_rB_s$ [M = Cu^{II}, H = H$^+$, A = aminosulfonate ligand, B = L-histidinate(1−)], at 25 °C and ionic strength 0.15 mol dm^{-3} NaCl

				Log β_{pqrs}		
p	*q*	*r*	*s*	A = amsa	A = absa	
0	1	1	0	8.47 (0.04)[a]	8.26 (0.04)	
0	2	1	0	0.6 (0.08)	0.4 (0.1)	
1	1	0	2	24.006[b]	23.65 (0.02)	24.006[b]
1	0	0	2	18.453[b]	18.88 (0.01)	18.453[b]
1	0	1	1	15.85[c] (0.02)	17.01 (0.02)[d]	17.03 (0.02)[e]

[a] 30 Values. [b] Values taken from ref. 10. [c] Residual error $R = 5.37 \times 10^{-7}$. [d] $R = 1.5 \times 10^{-6}$. [e] $R = 2.01 \times 10^{-6}$.

much better (*ca.* 9.0) and close to the values reported here for amsa and absa.

The protonation constants for L-histidine were taken from the literature.[11]

Cu^{II}–L-*histidine*–HA *Equilibria: Potentiometry*.—Analysis of the titration results for both ligands over the pH range 4–8 showed just three complexes to be present $[Cu(HHis)(His)]^+$, $[Cu(His)_2]$ and the ternary species [Cu(His)A] [His = L-histidinate(1 −)].

Any $[CuA_2]$ species was rejected by the fitting routine. As previously[12] two different iteration procedures (BETACALC3 and LEASK3) were used to verify that the final outcome corresponded to a true mathematical minimum. Initially the stability constants for the Cu–histidine complexes were fixed at literature values.[11] Subsequently, these were allowed to float to be determined from the titration data; gratifyingly, as Table 1 shows, this made only a small difference to the final constants obtained and had no effect on the number or type of species found. Speciation profiles for the systems (Cu–His–HA = 1:2:6) are shown in Figs. 1 and 2.

Spectroscopic Analysis.—For confirmation of the species proposed from the above mathematical fit, an analytical spectroscopic examination of the ternary solutions was carried out. The pH dependence of the visible absorption spectrum of a Cu–His–Hamsa solution is shown in Fig. 3, a similar dependence was observed with absa. In both cases the spectra above pH ≈ 7 were consistent with $[Cu(His)_2]$ being the single species present, in agreement with the potentiometric results (Figs. 1 and 2). Using the three species and their stability constants derived from the titration data, an acceptable fit was achieved with the amsa solution spectra. The spectroscopic values obtained for the three species are shown in Table 2. With the absa spectra, however, an acceptable fit was achieved assuming the presence of $[Cu(His)_2]$ and [Cu(His)(absa)] only because of the low concentrations of the $[Cu(HHis)(His)]^+$ species in this system over the pH range examined (see Fig. 2).

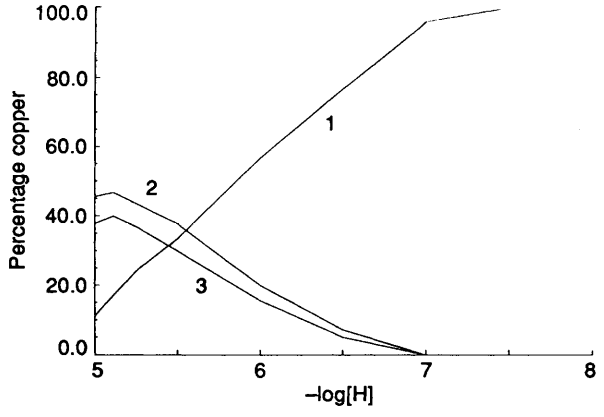

Fig. 1 Species distribution profile for the system Cu^{II}–His–Hamsa (1:2:6 ratio), in 0.15 mol dm^{-3} NaCl at 25 °C. Output from PLOT3: 1, $[Cu(His)_2]$; 2, $[Cu(HHis)(His)]^+$; 3, [Cu(His)(amsa)]

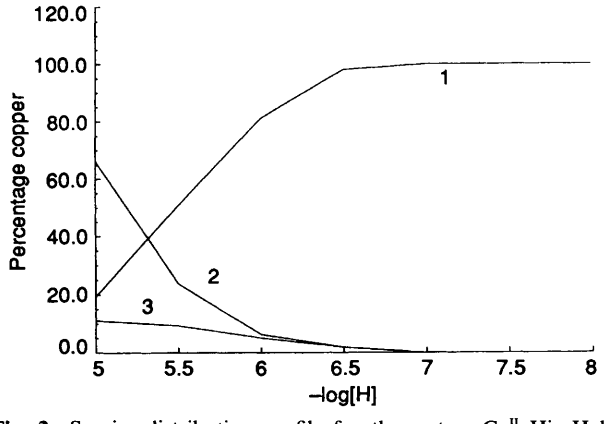

Fig. 2 Species distribution profile for the system Cu^{II}–His–Habsa (1:2:6 ratio), in 0.15 mol dm^{-3} NaCl at 25 °C. Output from PLOT3: 1, $[Cu(His)_2]$; 2, [Cu(His)(absa)]; 3, $[Cu(HHis)(His)]^+$

Conclusion

The large stability constants for the two ternary complexes imply that in both cases the aminesulfonate is binding as a bidentate ligand, most likely through N,O co-ordination. The larger value for the absa chelate shows a greater thermodynamic stability of the six-membered chelate ring as compared to the five-membered amsa system. This is supported by examination of space filling models (Ealing CPK atomic models) which show a very rigid five-membered chelate ring. This finding is also reminiscent of chelates formed from linear polyamines in which an extra carbon atom in the backbone produces less ring strain and hence enhanced stability for the six-membered rings (see ref. 13 and refs. therein). This difference in stability is also reflected in the shift to lower wavelength of the Cu d–d absorption for the [Cu(His)(absa)] complex.

Despite the favourable stability constants the species distribution profiles, Figs. 1 and 2, show that neither ligand, even in excess, at physiological pH is likely to compete with histidine for any available Cu ions. However, an important observation is the predominance of the ternary complexes at lower pH, particularly [Cu(His)(absa)]. If the sulfonate groups were thus part of some larger dentate ligand, the enhanced stability should lead to such a molecule being a potential chelate at the low pH of the digestive system.

Experimental

Materials.—The compounds Hamsa (BDH) and Habsa (Fluka) were recrystallised twice from water, dried at 60 °C and

Table 2 Visible absorption maxima (λ_{max}) and molar absorption coefficients (ε) of the Cu^{II} complexes computed from measured spectra at 25 °C and in aqueous 0.15 mol dm^{-3} NaCl solution

Complex	λ_{max}/nm	ε/dm^3 mol^{-1} cm^{-1}
$[Cu(His)_2]$	640	85
$[Cu(HHis)(His)]^+$	615	63*
[Cu(His)(amsa)]	630	25
[Cu(His)(absa)]	615	42

* This complex not found in the Cu–His–absa spectra.

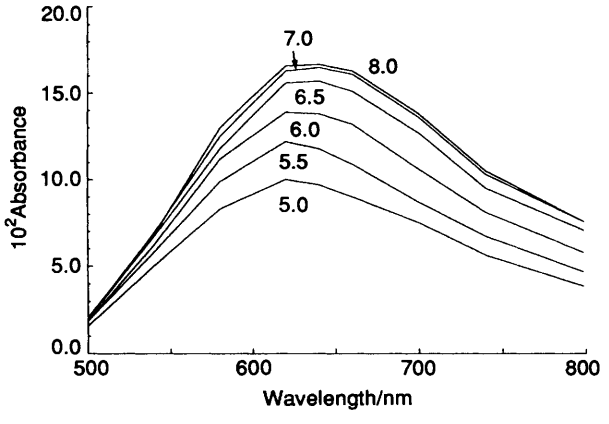

Fig. 3 pH Dependence of the visible absorption spectrum of the ternary Cu^{II}–His–Hamsa system (1:2:6 ratio) in 0.15 mol dm^{-3} NaCl at 25 °C

Table 3 Concentrations of reactants* in titration solutions for ligand protonation measurements ($\times 10^{-3}$ mol dm^{-3})

	Hamsa	HCl		Habsa	HCl
Set A1	0.025	0.100	Set B1	0.017	0.100
	0.025	0.100		0.031	0.125
Set A2	0.015	0.025	Set B2	0.010	0.035
	0.020	0.025		0.015	0.035
	0.025	0.025		0.017	0.100

* All solutions contained 0.15 mol dm^{-3} NaCl.

Table 4 Concentrations of reactants* in titration solutions for metal complex measurements ($\times 10^{-3}$ mol dm^{-3}); A = Hamsa, B = L-histidine

Titration No.	Cu^{II}	A	B	HCl
Set 3, metal variation series				
1	2.00	12.00	4.00	20.00
2	1.50	12.00	4.00	20.00
3	1.00	12.00	4.00	20.00
4	0.50	12.00	4.00	20.00
Set 4, ligand A variation series				
5	2.00	4.00	4.00	12.00
6	2.00	8.00	4.00	16.00
7	2.00	12.00	4.00	20.00
8	2.00	16.00	4.00	24.00
Set 5, ligand B variation series				
9	2.00	12.00	4.00	20.00
10	2.00	12.00	6.00	24.00
11	2.00	12.00	8.00	28.00
12	2.00	12.00	10.00	32.00

* All solutions contained 0.15 mol dm^{-3} NaCl.

stored over silica gel (Found: C, 11.05; H, 4.40; N, 12.85. CH_5NSO_3 requires C, 10.80; H, 4.50; N, 12.60%. Found: C, 40.85; H, 4.00; N, 8.60. $C_6H_7NSO_3$ requires C, 41.62; H, 4.05; N, 8.10%). L-Histidine (Fluka Biochem. purity) was stored over silica gel, $CuCl_2 \cdot H_2O$ and all other reagents were BDH AnalaR grade. All water was distilled and deionized.

Potentiometric Titrations.—These were carried out in 0.150 mol dm^{-3} NaCl solutions at 25.00 ± 0.05 °C. The instrumentation, titration procedure and analyses of the stock Cu^{II} and NaOH solutions were as previously described.[7,9] The electrode system was calibrated before and after each titration by measuring the emf changes of HCl *versus* NaOH titrations over the pH range 2–11. Each titration was repeated until an overall reproducibility of ±0.01 cm^3 of added titrant was obtained. For the determination of the protonation constants two sets of solutions were used to avoid excessive dilution; set A (see Table 3) was for the −log [H] range *ca.* 1–2.5 and set B for the range *ca.* 2–11. The solution concentrations for the metal complex determinations are given in Tables 4 and 5.

Computation of the equilibrium constants was *via* the suite of programs PLOT3, GUESS3, LEASK3 and BETACALC3, originally written by Sarkar and Kruck[8] and subsequently modified.[7,9]

Table 5 Concentrations of reactants* in titration solutions for metal complex measurements ($\times 10^{-3}$ mol dm^{-3}); A = Habsa, B = L-histidine

Titration No.	Cu^{II}	A	B	HCl
Set 6, metal variation series				
13	2.00	12.00	4.00	20.00
14	1.50	12.00	4.00	20.00
15	1.00	12.00	4.00	20.00
16	0.50	12.00	4.00	20.00
Set 7, ligand B variation series				
17	2.00	12.00	4.00	20.00
18	2.00	12.00	6.00	24.00
19	2.00	12.00	8.00	28.00
20	2.00	12.00	10.00	32.00
Set 8, ligand A variation series				
21	2.00	8.00	4.00	16.00
22	2.00	12.00	4.00	20.00
23	2.00	16.00	4.00	24.00
24	2.00	20.00	4.00	28.00

* All solutions contained 0.15 mol dm^{-3} NaCl.

UV/VIS Spectroscopy.—Absorption spectra were recorded with a Perkin-Elmer 555 spectrophotometer with the cell block thermostatted at 25.0 ± 0.1°C. The reactant concentrations used were the same as those of the mid-point titration curves, *i.e.* solution 1 (same as 7 and 9) in Table 4 for amsa and solution 13 (= 17 and 22) in Table 5 for absa. The −log [H] values were adjusted with NaOH within the range 5.0–8.0. Deconvolution of the measured spectra was carried out as previously[9,13] using the programs GUESS3 and LEASK3.

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