The binding ability of famotidine, the antiulcerogenic agent. Ternary complexes with histidine and histamine with copper(II)

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Abstract

Potentiometric and spectroscopic data have shown that the H_2 histamine antagonist famotidine is a very effective ligand able to compete with naturally occurring low molecular mass chelating agents like histidine and histamine. Ternary complexes of Cu(II) with famotidine, and either histidine or histamine were found to be very stable species, forming at low pH and being the major components in equilibrium mixture over almost the entire pH range.

Introduction

Recent work on the binding of famotidine (**1**), 3-[[[2-[(aminoiminomethyl)amino]-4-thiazolyl]methyl]-thio]-*N*-(aminosulfonyl) (LH), has shown that this potent inhibitor of the histamine H_2 receptor site [1] is also an excellent chelating agent for Cu(II) ions [2]. The sulfamide terminal is an effective binding centre in acidic solutions (pH < 2), the thiazole ring nitrogen and the vicinal thioether sulfur donors coordinate at intermediate pH and the vicinal guanidine is a potential donor centre at high pH. As a result, famotidine would be expected to be a very effective competitor for natural low molecular mass chelating ligands such as histidine (His), $NH_2CH(CH_2 \cdot C_3N_2H_3)COOH$ or histamine (Hstm) $NH_2CH_2CH_2C_3N_2H_3$, and would be expected to influence the homeostasis of essential metal ions in biological fluids when administered as a drug. In order to investigate this possibility, spectroscopic and potentiometric studies have been performed on the ternary systems famotidine with two of its natural competitors, His and Hstm.

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Experimental

Famotidine was obtained as a gift from Therapicon (Italy) and used without further purification. Histidine and histamine were used as obtained from Fluka. Purity of the ligands was checked by potentiometry and chromatography.

Spectroscopic studies

EPR spectra were recorded on a Varian E-9 or a Radiometer SE spectrometer at the X-band frequency (9.3 GHz) at 120 K. Absorption spectra were performed on Uvikon 810P and Beckman UV 5240 spectrophotometers. Circular dichroism spectra were recorded on a Jasco J-600 spectropolarimeter. Solutions containing either 0.005 or 0.0025 mol dm^{-3} of Cu^{2+} with metal to ligand ratio of 1:1:1 (Cu:L:L'; L = famotidine, L' = histidine or histamine) were used for the spectroscopic measurements.

Potentiometric studies

Titration data were collected as 25 °C with a Tacussel ISIS 2000 pH-meter using total volumes of 3 cm^3 under the following experimental conditions: Cu(II) concentration 0.001 mol dm^{-3}; ionic strength 0.10 mol dm^{-3}

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(KNO_3); molar ratio when studying ternary complexes, 1:1:1, when studying binary complexes, 1:2 or 1:3; pH range for complexation, 2.0–10.4; method, pH-metric titration using micro-combined glass–calomel electrode (Radiometer), calibrated in concentration using daily titrations with HNO_3 [3]; number of titrations three per ligand (average of 40 data points per titration); temperature 25 °C; method of calculations, SUPERQUAD [4].

Standard deviations quoted were computed by SUPERQUAD and refer to random errors only. They give, however, a good indication of the importance of the particular species in equilibrium.

Using SUPERQUAD it was possible to check the purity of the ligands by refining on total ligand concentrations when determining protonation constants. Purity was found to be at least 99%.

Results and discussion

Copper(II) complexes

Histidine (His) and histamine (Hstm) are both very effective ligands for many transition metal ions because they contain both imidazole and amino nitrogen donors in suitable positions for chelate formation, His being the more potent chelating agent because it can act as a tridentate ligand through the carboxyl group [5–7]. As a result binding to Cu(II) starts as low as pH 3 with His or pH 4 with Hstm, and the free concentration of Cu(II) is reduced to less than 1% by pH 5 in the presence of a two-fold excess of ligand. Famotidine is an even more effective ligand, particularly at low pH, and the coordination equilibria in its ternary systems are distinctly different from those observed for the binary complexes. Starting around pH 2 the binary complexes $Cu(Fam)^{2+}$ and $Cu(Fam)_2^{2+}$ are the first to form (comparable to the equilibria in the Cu(II)–Fam binary system [2]) but around pH 3 in solutions of Cu(II):Fam:His (or Hstm) of 1:1:1 ternary complexes are formed with the result that the Cu(II)–Fam binary complexes never become the dominant species.

Above pH 4 the ternary complexes Cu(Fam)L, $Cu(FamH_{-1})L$ and $Cu(FamH_{-2})L$ (where L = His or Hstm, charges omitted for simplicity) are formed sequentially as shown in Fig. 1. These ternary complexes were identified clearly from the potentiometric data. When the species were incorporated in the equilibrium the statistics of the fit between measured and calculated potentials was good throughout the titration range with a near-random distribution of residuals ($E_{calc} - E_{meas}$) and differences were always less than equivalent to 0.01 pH unit. If either of the ternary species was omitted from the equilibrium there was a dramatic deterioration in the goodness of fit, particularly in the pH range in which the ternary species would be anticipated.

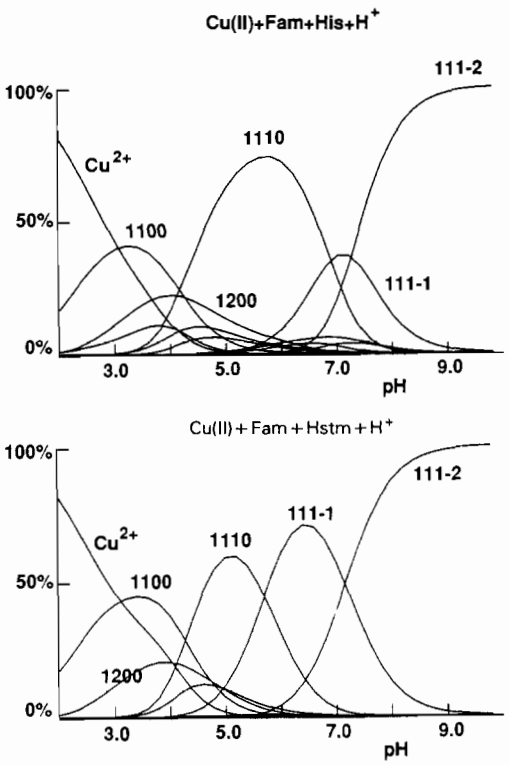

Fig. 1. Species distribution curves for Cu(II)–Fam–His and Cu(II)–Fam–Hstm systems. The metal ion concentration is 10^{-3} mol dm^{-3} and molar ratio is 1:1:1 (M:L:L').

The presence of ternary complexes is also reflected in the spectroscopic data summarized in Table 1. Since His is a chiral molecule CD spectroscopy may be used to establish whether it is bound directly to the Cu(II) ion, as d–d transitions in the CD spectra are only possible under these circumstances. In the Cu(II)–Fam–His system d–d transitions in the 670–690 region are present above pH 4 confirming Cu(II)–His binding, while the other parameters given in Table 1 clearly indicate that species formed in the binary solutions are distinctly different from those formed in ternary systems. EPR spectra give information on the number of coordinated nitrogen donors as well as the number of species formed. Their pH dependence corresponds closely to the species distribution curves calculated from pH-metric titrations (e.g. Fig. 1).

Stability constants of binary complexes of Cu(II) with His and Hstm are distinctly different (see Table 2) because His coordinates as a tridentate ligand while Hstm is only a bidentate ligand. In the ternary complexes, however, the stability constants are similar, suggesting that both ligands are coordinating in the same manner, i.e. as bidentate ligands bonded through the imidazole and amino nitrogens. Histamine-like coordination of His in Cu(II) complexes (including ternary systems) is already well established [6–9].

The coordination pattern of Fam in its ternary complexes is closely similar to that found in its binary complexes. For example in the Cu(Fam)L complexes the binding of the Fam molecule is via the N^{15} and N^{18} nitrogens at the sulfonamide terminal (see Structure 1). As the pH is increased the next proton to be removed from the ternary complex must be from the thiazole nitrogen to form an additional Cu–N bond in Cu(FamH$_{-1}$)L. Coordination of the thiazole ring nitrogen together with the N^{15} donor should facilitate binding of the S^{11} thioether sulfur to give two chelate rings, one six-membered ({N^{15}, S^{11}} and the other five-membered {S^{11}, N^{3}}). Coordination of sulfur to Cu(II) is confirmed by the absorption spectrum which shows a distinct although poorly resolved band around 450 nm corresponding to the S–Cu(II) charge transfer transition. The band is weaker than expected so suggesting a rather long Cu–S bond, which is characteristic for a thioether sulfur donor in an apical position [12, 13].

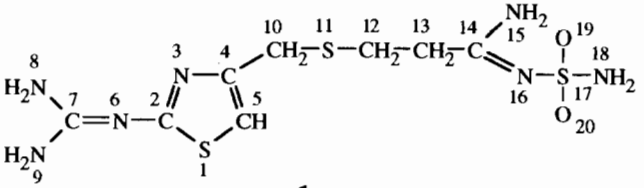

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TABLE 1. Spectroscopic data for binary Cu(II) complexes with His, Hstm and Fam and ternary complexes obtained in Cu(II)–His–Fam and Cu(II)–Hstm–Fam systems

Species	UV–Vis		CD		EPR	
	λ (nm)	ε	λ (nm)	(Δε)	$A_{\parallel}$	$g_{\parallel}$
His						
M L′ H						
1 1 0	671[a]	40	726[a]	(+0.146)	161	2.298
			551[a]	(−0.046)		
			321[b]	(−0.091)		
1 2 0	639[a]	56			171	2.248
Hstm						
M L′ H						
1 1 0	670[a]	36			170	2.295
	320sh[b]	92				
1 2 0	595[a]	98				
Fam						
M L H						
1 2 0	630[a]	130			190	2.220
	312[b]	2640				
1 2–1	620[a]	150			178	2.216
	455[c]	110				
1 2–2	600[a]	160			182	2.212
	440[c]	175				
1 2–3	575[a]	180			172	2.219
	440[c]	190				
	330sh[b]	1200				
His–Fam						
M L′ L H						
1 1 1 0	630[a]	118	673[a]	(+0.219)	186	2.236
	320[b]	1262				
1 1 1–1	612[a]	123	692[a]	(+0.224)	192	2.225
1 1 1–2	605[a]	163	686[a]	(+0.286)	182	2.235
	447[c]	92				
Hstm–Fam						
M L′ L H						
1 1 1 0	628[a]	112			183	2.221
	317[b]	2000				
1 1 1–1	613[a]	118			181	2.215
1 1 1–2	598[a]	150			194	2.223
	442sh[c]	93				

[a]d–d transitions. [b]N ⇒ Cu(II). [c]S ⇒ Cu(II) charge transfer transitions.

TABLE 2. Stability constants (log β) for binary and ternary complexes formed in Cu(II)–famotidine–histidine and Cu(II)–famotidine–histamine solutions

Species	log β		log X[a]
His	this work		
M L′ H			
0 1 3	16.88(0.01)	16.88[b]	
0 1 2	15.17(0.01)	15.16	
0 1 1	9.12(0.01)	9.11	
1 1 0	10.21(0.09)	10.16	
1 2 0	18.45(0.01)	18.11	
1 1 1	14.35(0.01)	14.11	
1 2 1	24.17(0.01)	23.81	
1 2 2	27.30(0.03)	27.20	
Hstm			
M L H			
0 1 2	15.10(0.01)	15.11[c]	
0 1 1	9.16(0.01)	9.06	
1 1 0	9.66(0.01)	9.58	
1 2 0	16.10(0.01)	16.06	
1 2 1	21.82(0.01)	21.79	
Fam			
M L H			
0 1 1		6.86[d]	
1 1 0		7.27	
1 2 0		14.03	
1 2–1		7.79	
1 2–2		0.90	
His–Fam			
M L′ L H			
1 1 1 0	17.44(0.03)		2.38
1 1 1–1	10.56(0.06)		
1 1 1–2	3.31(0.04)		
Hstm–Fam			
M L′ L H			
1 1 1 0	17.06(0.04)		3.97
1 1 1–1	11.36(0.06)		
1 1 1–2	4.20(0.07)		

[a]$\log X = (\log K^{CuL'}_{CuL'L} - \log K^{CuL}_{CuL_2}) + (\log K^{CuL}_{CuLL'} - \log K^{CuL'}_{CuL'_2})$, ref 10. [b]Ref. 11. [c]Ref. 9. [d]Ref. 2.

Binding of Cu(II) by Fam in the pH range 6–7 using the $\{N^{15}, S^{11}, N^{3}\}$ donor set gives a very stable complex. The $Cu(FamH_{-2})L$ complex becomes the major species above pH 7. In this complex the guanidine nitrogen also coordinates to the metal ion and it is interesting to note that dissociation of the guanidine proton in the ternary complexes takes place about a pH unit lower than in the Cu(II)–Fam binary system.

Evaluation of the stabilization of ternary complexes based upon the data obtained for the binary species and using the quantity $\log X$ (defined in Table 2) indicates that stabilization is higher for ternary complexes containing Hstm than those containing His. This increased stabilization must result from lower steric effects when Hstm rather than His is in the ternary complex. The stronger steric effects are also reflected in the EPR parameters obtained for the $Cu(FamH_{-2})L$ complexes. The formation of the His ternary complex leads to a considerable decrease of $A_{\parallel}$ and increase of $g_{\parallel}$ when compared to Hstm ternary species (Table 1). These changes indicate stronger tetrahedral distortion in the His complex when the guanidine nitrogen is involved in the metal ion coordination. It may support multidentate (more than bidentate) coordination of Fam to the Cu(II) ion in ternary systems. It should be mentioned that an X-ray structure obtained of a Cu(II) complex with a close derivative of famotidine (cimetidine) has shown such a possibility for binary complexes [14].

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