Famotidine, the New Antiulcero-Genic Agent, a Potent Ligand for Metal Ions

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ABSTRACT

Potentiometric, polarographic, and spectroscopic results obtained for Cu^{2+} and Ni^{2+}-famotidine systems clearly indicated that this anti-ulcerogenic drug is a very potent chelating agent able to coordinate cupric ion that was at pH below 2. This drug exhibits excellent histamine H_2 receptor blocking effects and its effective coordination to metal ions may have significant biological implications. Famotidine is found to be a very effective ligand for Ni^{2+} ions also.

INTRODUCTION

Famotidine (**1**), 3-[[[2-[(aminoiminomethyl)amino]-4-thiazolyl]methyl]thio]-N-(aminosulfonyl), is known as an anti-ulcer drug having excellent histamine H_2 receptor blocking effect similar to cimetidine (**2**), the widely used histamine H_2 antagonist [1]. The latter drug as well as histamine itself is a rather effective ligand and both of them may potentially interact with the essential metal ions in blood plasma or other tissues.

Cu^{2+} ion was shown to increase dramatically the cimetidine binding to imidazole receptors located in rat brain [2]. The computer-simulated distribution of the involved complexes does not show, however, any important impact of this drug on the bioavailability of essential metal ions [3, 4]. The drug is not very competitive for naturally occurring ligands. It is, however, likely that Cu^{+} may form more potent complexes with cimetidine than Cu^{2+} [5, 6]. Recent work [6] has shown that Cu-cimetidine complexes exhibit unusually high superoxide dismutase-like activity. Thus, the biological implications of the metal drug complexes may be quite important although still unknown. Famotidine, which is

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a recent analog of cimetidine serves different, potentially more effective donor sets including guanidine moiety bound to thiazole ring. Therefore, the complexes of this drug with essential metal ions may be of even higher biological interest than those with cimetidine.

In this work we present the results showing the thermodynamic and structural features of the Cu^{2+} and Nl^{2+} complexes with famotidine, which is found to be a much more effective ligand than cimetidine and can be competitive even for histamine and histidine.

EXPERIMENTAL

Famotidine was obtained as a gift from THERAPICON (Italy) and used without further purification. Its purity was checked by potentiometric titrations and chromatography.

Spectroscopic Studies

EPR spectra were recorded on a VARIAN or a RADIOMETER SE spectrometer at X-band (9.3 GHz) at 120 K. Absorption spectra were performed on a UVIKON 810P or a BECKMAN UV 5240 spectrophotometer. Solutions containing 0.005 or 0.0025 mol dm^{-3} of Cu^{2+} with metal-to-ligand ratio of 1:2, 1:4, and 1:5 were used for the spectroscopic measurements. In the case of Ni^{2+} the metal concentration was 0.0025 mol dm^{-3} and metal-to-ligand ratio was 1:5.

Potentiometric Studies

The titration data were collected at 25°C with TACUSSEL ISIS 2000 pH meter using total volumes of 3 cm^3. Changes of pH were followed by RADIOMETER combined glass-calomel electrodes calibrated for H^+ activity. The relations between activities and concentrations were calculated daily by titration with HNO_3 [7]. All titrations were carried daily by titration with HNO_3 [7]. All titrations were carried out under argon and constant ionic strength 0.1 M (KNO_3). The 0.1 M solution of $CuCl_2$ or $NiCl_2$ were used as the stock solutions for metal ions. The metal concentration was fixed to 0.001 mol dm^{-3} and the metal-to-ligand molar ratios were 1:2, 1:3, and 1:4 for Cu^{2+} and 1:4, 1:5, and 1:7 for Ni^{2+}, respectively.

The calculations of the stability constants were performed with a SUPERQUAD computer program [8] which allows for the simultaneous refinement of stability constants together with total ligand and total hydrogen concentrations.

The standard deviations quoted were computed by SUPERQUAD and referred to random errors only. These give, however, a good indication of the importance of a particular species in the equilibrium.

Polarographic Measurements

Since the complex formation in the Cu^{2+}-famotidine system was observed at very low pH, the polarographic measurements were carried out to verify the potentiometric evaluations of the stabilities and stoichiometries of the species formed at a lower pH range.

Polarographic experiments were performed by using a PA-4 polarographic analyzer interfaced with a SMDE-1 cell stand and a three-electrode cell config-

uration. A hanging mercury drop electrode (HMDE) was used as a working surface, a platinum wire as an auxiliary electrode, and a saturated calomel reference electrode.

The differential pulse polarographic (DPP) measurements on HMDE were carried out with a pulse amplitude 50 mV, a scan range from +0.150 to −0.500 V at scan rate 10 mV/s.

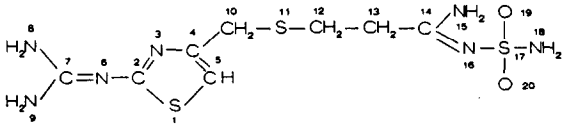
Aqueous solutions containing 5×10^{-5} mol dm^{-3} of metal ion were titrated with famotidine (5×10^{-3} mol dm^{-3}). The metal-to-ligand ratio varied from 1:1 to 1:20. The measurements were carried out at 20°C over pH range 2.0–5.0 with ionic strength 0.4 M of Na_2SO_4. This electrolyte and its concentration were experimentally chosen to obtain the best quality of polarographic features of the studied system. The solutions were purged with argon for 10 min before each experiment.

The stability constants from the polarographic data were evaluated according to the De Ford-Hume method [9].

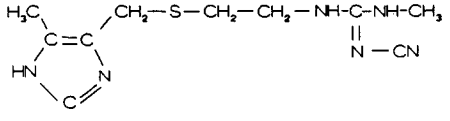
RESULTS AND DISCUSSION

Famotidine (HL) contains one dissociable proton with pK = 6.86 which most likely corresponds to the protonation of the thiazole ring nitrogen. This value is close to that found for the imidazole ring of cimetidine (pK = 6.70) [3].

According to the polarographic, potentiometric, and spectroscopic results, the Cu^{2+} ion coordination with famotidine begins already at pH below 2.0 (Table 1, Fig. 1a). This indicates very strong binding ability of famotidine. The potentiometric and polarographic data show that at pH below 6 there are two major species formed, CuL and CuL_2. The CuL complex reaches the highest concentration (50% of total Cu^{2+}, Fig. 1a) at pH around 3, while the CuL_2 is a dominant species (80% of total metal) at pH 5. Formally, both of these complexes are also found in the system with cimetidine. Their stabilities, however, are considerably lower than those of famotidine complexes (3 and 6 orders of magnitude, respectively, Table 1). It is then evident that the species



(1)



(2)

SCHEME 1

TABLE 1. Stability Constants (log β) for H^+, Cu^{2+}, and Ni^{2+} Complexes with Famotidine

Species M L H	Famotidine	Cimetidine[3]
0 1 1 (LH)	6.86(0.01)	6.70
Cu^{2+}		
1 1 0 (CuL)	7.27(0.02) 7.37(0.02)*	4.16
1 2 0 (CuL_2)	14.03(0.01) 13.95(0.02)*	8.30
1 3 0 (CuL_3)		10.70
1 2 −1 (CuL_2H_{-1})	7.79(0.01)	0.546
1 2 −2 (CuL_2H_{-2})	0.90(0.01)	
1 2 −3 (CuL_2H_{-3})	−6.92(0.01)	
Ni^{2+}		
1 1 0 (NiL)	3.46(0.05)	
1 1 −2 ($NiLH_{-2}$)	−13.20(0.04)	

*Stability constants obtained from polarographic measurements.
Standard deviations are given in parentheses.

found for famotidine and cimetidine differs completely from each other. Since the proton formation constants of the thiazole and imidazole ring nitrogens are very close to each other it is rather clear that the metal ion coordination modes of both ligands must be different. According to the studies in solution and solid state [3, 6, 10] the binding site in cimetidine is centered at imidazole nitrogen and vicinal thioether sulphur atom. Such coordination is effective at pH well above 4 and cannot be the case in the CuL and CuL_2 complexes of famotidine. The involvement of very basic (pK > 12) guanidine nitrogens is likely, but only at higher pH (vide supra). Thus, the sulfamide terminal seems to be the only likely binding site for cupric ions in both the CuL and CuL_2 complexes. The possibility of formation of the 6-membered chelate ring by two nitrogens N^{15} and N^{18} (1), (atom numbering taken from Ref. 14), seems to be the reasonable assumption. The terminal nitrogen bound to sulfate moiety is expected to be the anchor donor for metal ion in acidic pH. The closing of the chelate ring with the vicinal amide N^{15} leads to the formation of the very stable CuL and CuL_2 complexes. Such coordination leads to release of one or two protons, respectively. Thus, the CuL and CuL_2 species should be written formally as $Cu(LH)H_{-1}$ and $Cu(LH)_2H_{-2}$, respectively, since the thiazole dissociable proton remains at its binding site.

The spectroscopic data support the discussion based on the potentiometric and polarographic data. The well resolved spectra could be observed only for $Cu(LH)_2H_{-2}$ which predominates at pH around 5. The absorption spectra exhibit the d-d transitions centered at 630 nm with rather high absorbance

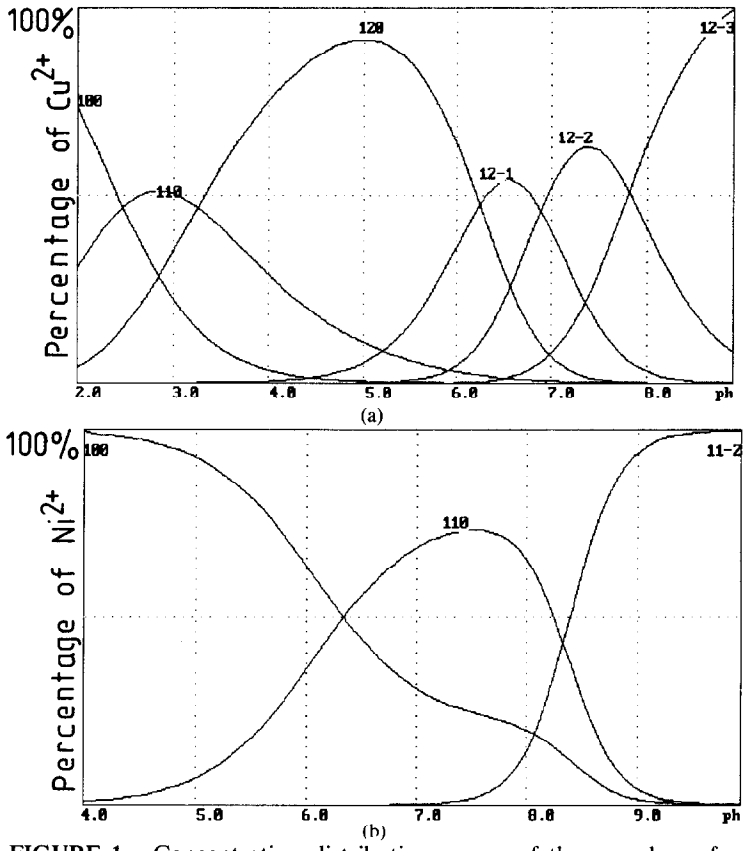


FIGURE 1. Concentration distribution curves of the complexes formed in the Cu^{2+}-famotidine (a) and Ni^{2+}-famotidine (b) solutions as a function of pH; $c_{M^{2+}} = 10^{-3}$ mol dm^{-3} and $c_{FAM} = 2 \times 10^{-3}$ mol dm^{-3}p.

($\epsilon = 140$). These transitions are strongly shifted towards higher energies when compared to the aquaion species (830 nm). This clearly indicates the involvement of the nitrogen donors in metal ion coordination [11]. The formation of the first two complexes leads also to the charge-transfer band observed at 315 nm which may be attributed to the amide-like nitrogen (e.g., N^{15}) to Cu^{2+} transition [11].

The increase of pH above 6 results in deprotonation of the above discussed complexes and the formation of the CuL_2H_{-1} and CuL_2H_{-2} species. Their spectroscopic features change drastically when compared to the lower pH complexes (Table 2). The d-d transition shifts again to higher energy and the new band appears at 440 nm. The transition at 440 nm may be attributed to the charge-transfer transition from the apically bound thioether sulphur [12, 13]. Thus, the formation of the CuL_2H_{-1} and CuL_2H_{-2} species changes the binding mode of metal ion. The involvement of sulphur donor indicates that the thiazole nitrogen is also bound to cupric ion as in the case of cimetidine containing systems [10]. The molecular model considerations suggest that metal ion binds to N^{15} and thiazole nitrogens while thioether sulphur coordinates in apical positions. Such coordination of sulphur leads to creation of two stable

TABLE 2. Spectroscopic Data for the Complexes Obtained for the Cu^{2+} and Ni^{2+} Famotidine Complexes

Species	Absorption Spectra λ[nm] (ε)	EPR g//	EPR A//[gauss]
CuL_2	630 (130)[a]	2.220	190
	312 (2640)[b]		
CuL_2H_{-1}	620 (150)[a]	2.216	178
	455 (110)[c]		
CuL_2H_{-2}	600 (160)[a]	2.214	180[e]
	440 (175)[c]		
CuL_2H_{-3}	575 (180)[a]	2.219	172
	440 (190)[c]		
	sh330 (1200)[d]		
NiL (octahedral)	875 (16)[a]		
	575 (12)[a]		
	sh360 (23)[a]		
$NiLH_{-2}$ (planar)	sh505 (111)[a]		
	417 (184)[a]		

[a]d-d Transition.
[b]$N \rightarrow Cu^{2+}$ Charge-transfer transition.
[c]$S \rightarrow Cu^{2+}$ Charge-transfer transition.
[d]$N \rightarrow Cu^{2+}$ Charge-transfer transition.
[e]Spectra are overlapped and parameter estimation is not very precise.

chelate rings, 6- and 5-membered, respectively. The involvement of the $\{N^{15}, N^3, S\}$ donor set explains also the number of released protons from CuL_2H_{-1} and CuL_2H_{-2} molecules (i.e., from the N^{15} and thiazole nitrogens).

At pH above 8 the dominant complex is the CuL_2H_{-3} species. Its EPR parameters are different than those of the complexes discussed above ($A_{\|}$ parameter decreases from 190 to 172 gauss, Table 2) and the d-d transition shifts to 575 nm. These spectral changes indicate the involvement of additional nitrogen of guanidine moiety in the coordination sphere of Cu^{2+} ion. This strongly basic nitrogen substitutes much more acidic terminal sulfamide nitrogen donor. The stoichiometry of CuL_2H_{-3} species requires, however, the simultaneous binding of deprotonated N^{15} donor as well as the deprotonated guanidine nitrogen in one of the coordinated ligands. The presence of the 440 nm band indicates that sulphur is bound also in the CuL_2H_{-3} species. Thus, the latter species should be presented formally as $Cu(LH_{-1})(LH_{-2})$. The coordination modes of these two bound ligands are $\{N^{15}, S, N^3\}$ and $\{N^{15}, N^3, N^8\}$, respectively. It is clear that the copper complex remains tetragonal, one donor of each bound ligand should be coordinated in the apical position. The distinct lowering of the $A_{\|}$ value may derive from this rather strong apical coordination to cupric ion [15].

The results presented above clearly show that famotidine is a very effective ligand for copper ions. The stability constants are considerably higher than those of cimetidine. Therefore the biological implications of famotidine involvements in metal ion binding can be much more likely than those suggested for cimetidine. The binding ability of sulfamide moiety as the anchor group in acidic solutions is quite unique especially when inserted in the system containing other powerful donor sets.

Ni^{2+} ions form only two species, NiL and $NiLH_{-2}$. The spectroscopic data indicate that the former complex is octahedral, while the latter one exhibits the strong d-d transitions around 505 and 417 nm typical for the planar species (Table 2, Fig. 1b). The change of the octahedral geometry (NiL) into considerably more stable square-planar structure ($NiLH_{-2}$) is the main cause of the simultaneous release of two protons from the NiL complex at pH above 7.5. The same coordination pattern is observed for the peptide ligands when two or three consecutive amide protons are released simultaneously and the planar complex is formed [11, 16].

Ni^{2+} forms only equimolar complexes which are considerably weaker than respective cupric species (Table 1).

The coordination of Ni^{2+} begins at pH above 5 indicating the involvement of the thiazole nitrogen rather than the sulfamide terminal. The stability constant of the NiL species ($\log \beta = 3.46$) is slightly higher than that of respective imidazole complex ($\log \beta = 2.89$ [17]) and it suggests monodentate nitrogen coordination of the thiazole ring in this complex.

The formation of the planar $NiLH_{-2}$ species needs the involvement of at least three nitrogen donors of famotidine [11, 16]. It is likely that the binding mode in the latter complex is similar to that discussed above, i.e., involving the $\{N^3, N^8, N^{15}\}$ donor set.

CONCLUSIONS

Famotidine is found to be a very effective chelating agent for cupric ions because of sulfamide and guanidine moieties which are involved in the metal ion coordination at acidic and at basic media, respectively. The involvement of the thiazole ring nitrogen is critical at the intermediate pH range allowing coordination of both terminals to Cu^{2+} ion. Therefore, it is likely that the presence of famotidine in biological systems may considerably influence the behavior of Cu^{2+} ions in physiological fluids.

Thiazole is a basic binding site for Ni^{2+} ions and although this coordination is not as effective as in the case of Cu^{2+} the planar complex formed at pH above 8.0 is very stable.

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