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Complexes of the Histamine H_2-Antagonist Cimetidine with Divalent and Monovalent Copper Ions

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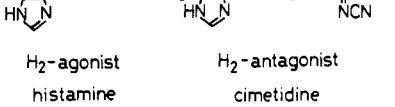
The histamine H_2-antagonist cimetidine (L) forms 1:1 and 2:1 complexes with Cu(II) ion in aqueous solutions. The complexation constants $K_{Cu^{II}L}$ = (3.02 ± 0.05) × 10^4 M^{-1} and $K_{Cu^{II}L_2}$ = (2.35 ± 0.05) × 10^4 M^{-1} are determined by the pH-metric titration method. By comparison with greater $K_{Cu^{II}L}$ and $K_{Cu^{II}L_2}$ values of other biological ligands such as histamine and peptides, it is concluded that cimetidine is unlikely to bind with Cu(II) in vivo. The polarographic measurements have estimated the Cu(I/II) redox potential $E°$ of +0.42 V vs. NHE for the 1:1 cimetidine complex, which implies a likelihood of physiological reduction with ascorbic acid or hemoglobin. Indeed, a stable 1:1 Cu(I)-cimetidine complex has been isolated (as a very insoluble solid) on treatment of Cu(II)-cimetidine complexes with ascorbic acid. The stability of the Cu(I)-cimetidine complex is enormous in that it can survive in the presence of biological ligands. These results may indicate an important role of copper ion in the pharmacological activities of cimetidine as cuprous-cimetidine complexes in our bodies. Since its $E°$ value of +0.42 V vs. NHE is comparable to those of blue copper (type I) proteins and Cu-superoxide dismutase, cimetidine is promising as a new biomimetic ligand for interconversion of Cu(I) and Cu(II). The Cu-cimetidine complexes exhibit higher superoxide dismutase like activity than any previous complex, suggesting great biochemical and drug potentials for cimetidine as copper complexes.

Introduction

Cimetidine (trade name Tagamet) is a potent histamine H_2-receptor antagonist, which inhibits excessive acid secretion caused by histamine, and currently is in worldwide clinical use for treatment of peptic ulcers.[2,3] The drug is taken orally and absorbed in the intestine and reaches H_2-receptors via the bloodstream. Cimetidine, like histamine, is a potential chelating agent. Since micromolar levels of loosely bound Cu(II) ion are present in blood serum,[4] it may bind to the drug. Earlier, a polymeric 1:2 Cu(II)-cimetidine complex was isolated (as green crystals) from a pH 7.0 aqueous solution for an X-ray analysis.[5] On the basis of ^1H and ^{13}C NMR studies of the 1:2 complex in aqueous solution, the binding sites of cimetidine were proposed to be the imidazole-N and nitrile-N donors.[5] A 1:1 Cu(II)-cimetidine complex is likely to coexist in the solution, but it has not been verified. A quantitative study on the copper complexes of cimetidine has not been reported until now. Especially interesting, from a pharmacological point of view, is whether cimetidine can compete for Cu(II) ion against biological ligands such as serum albumin or amino acids.



H_2-agonist histamine

H_2-antagonist cimetidine

Recently the Cu(II) ion was demonstrated to dramatically increase the cimetidine binding to imidazole receptors located in rat brain.[6-8] Cu(II) ion has been implicated in the regulation of the cimetidine binding sites in the brain. Addition of ascorbic acid or dithiothreitol further enhances the cimetidine binding.[9] One hypothesis is that Cu(II) in the cimetidine complex may undergo reduction to Cu(I) to act as a more potent binding promoter than Cu(II).[9] We therefore felt it imperative to determine the reduction potential of the Cu(II)-cimetidine complex. Our study has revealed that the Cu(I)-cimetidine complex is indeed generated in the presence of ascorbic acid. Its oxidation potential $E°$ of +0.42 V vs. NHE is extremely high and more interestingly is nearly the same as the $E°$ value of bovine Cu-superoxide dismutase (SOD).[10-12] We have discovered that the copper-cimetidine complexes possess superoxide dismutase like activity much more strongly than any previously reported copper complex does.

Experimental Section

Materials. Cimetidine was purchased from Sigma Chemicals and purified by recrystallization from MeOH/MeCN. A stock solution of Cu(II) ion was prepared from analytical grade $Cu^{II}[ClO_4]_2$ and standardized by titration with the disodium salt of ethylenediaminetetraacetic acid (EDTA).[13] $Cu^{I}[NCCH_3]_4ClO_4$ was freshly prepared according to the literature method.[14] Other chemicals employed were of analytical grade and were used without further purification.

Synthesis of the 1:1 Cu(I)-Cimetidine Complex. Treatment of $Cu^{I}[NCCH_3]_4ClO_4$ (16.4 mg) with 2 equiv of cimetidine (25.2 mg) in 10 mL of 2% CH_3CN-H_2O in Ar for 1 h at 25 °C yields the 1:1 Cu(I)-cimetidine complex as a white precipitate (19.5 mg). Anal. Calcd for $C_{10}H_{16}N_6SCuClO_4 \cdot H_2O$: C, 27.72; H, 4.19; N, 19.39. Found: C, 27.92; H, 3.92; N, 19.49. The 1:2 Cu(II)-cimetidine complex was prepared as described in ref 5.

Potentiometric Measurements. The formation constants of Cu(II)-cimetidine complexes were determined by potentiometric acid-base titration (an Orion Research 811 digital pH meter) of cimetidine with a carbonate-free 0.200 M NaOH solution in the presence of Cu(II) ion. The titration data were treated by a computer-aided Schwarzenbach method.[15] The temperature was maintained at 25.00 ± 0.05 °C, and the ionic strength was adjusted to 0.20 M with $NaClO_4$. All -log $[H^+]$ values were estimated with a correction of -0.13 pH unit to the pH meter readings.[16] All solutions were carefully protected from air by a stream of argon prepurified with an alkaline pyrogallol solution. The electrode system was calibrated with pH 7.00 and 4.01 buffer solutions and checked by the duplicate theoretical titration curves of 4.00 × 10^{-3} M $HClO_4$ with 0.200 M NaOH solution at 25 °C and I = 0.20 M ($NaClO_4$).

Electrochemical Measurements. Cyclic voltammetry and dc polarography were performed with a Yanaco Polarographic Analyzer P-1100 system at 25.00 ± 0.05 °C and I = 0.20 M ($NaClO_4$ or $NaNO_3$). A three-electrode system was employed: a 3-mm glassy-carbon rod (grade GC-30, Tokai Electrode Co.) or a Yanagimoto dropping mercury elec-

(1) (a) Hiroshima University. (b) Hirosaki University.
(2) Brimblecombe, R. W.; Duncan, W. A. M.; Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Parsons, M. E. *J. Int. Med. Res.* **1975**, *3*, 86.
(3) Ganellin, C. R.; Parsons, M. E., Ed. *Pharmacology of Histamine Receptors*; John Wright & Sons: Bristol, U.K., 1982.
(4) Perrin, D. D.; Agarwal, R. P. *Met. Ions Biol. Syst.* **1973**, *2*, 167.
(5) Greenaway, F. T.; Brown, L. M.; Dabrowiak, J. C.; Thompson, M. R.; Day, V. M. *J. Am. Chem. Soc.* **1980**, *102*, 7782.
(6) Kendall, D. A.; Ferkany, J. W.; Enna, S. J. *Life Sci.* **1980**, *26*, 1293.
(7) Burkard, W. P. *Eur. J. Pharmacol.* **1978**, *50*, 449.
(8) Chansel, D.; Oudinet, J.-P.; Nivez, M.-P.; Ardaillou, R. *Biochem. Pharmacol.* **1982**, *31*, 367.
(9) Kawai, M.; Nomura, Y.; Segawa, T. *Neurochem. Int.* **1984**, *6*, 563.
(10) Malkin, R.; Malmstron, B. G. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1970**, *33*, 177.
(11) Free, J. A.; DiCorleto, P. E. *Biochemistry* **1973**, *12*, 4893.
(12) Lawrence, G. D.; Sawyer, D. T. *Biochemistry* **1979**, *18*, 3045.
(13) Schwarzenbach, G.; Flaschka, H. *Die Komplexometrische Titration*; Ferdinand Enke: Stuttgart, FRG, 1965.
(14) Hathaway, B. J.; Holah, D. G.; Postlethwaite, J. D. *J. Chem. Soc.* **1961**, 3215.
(15) Schwarzenbach, G.; Willi, A.; Bach, R. O. *Helv. Chem. Acta* **1947**, *30*, 1303.
(16) Davies, C. W. *Ion Association*; Butterworths: Washington, D.C., 1962.

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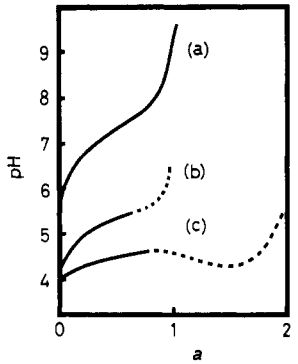


Figure 1. Potentiometric titration curves for L·$HClO_4$ (L = cimetidine) in the absence and presence of Cu(II) ion at 25 °C and I = 0.2 M ($NaClO_4$): (a) [total L] = 2.0×10^{-3} M only; (b) [total L] = [total Cu(II)] = 2.0×10^{-3} M; (c) [total L] = 1.1×10^{-2} M; [total Cu(II)] = 1.0×10^{-3} M. *a* for curve a is mole of base per mole of cimetidine; *a* for curves b and c is mole of base per mole of Cu(II) ion. The broken lines indicate precipitation of the 1:2 Cu(II)–cimetidine complex.

trode as the working electrode, a mercury pool as the counter electrode, and a saturated calomel reference electrode (SCE) with a potential of +241 mV vs. NHE at 25 °C. The cyclic voltammograms with scan rates of 10–100 mV s^{-1} were evaluated graphically.

Spectrophotometric Measurements. UV spectra were measured with a Shimazu UV-200S spectrophotometer at 25.0 ± 0.1 °C and I = 0.20 M ($NaClO_4$). IR spectra (KBr tablet) were obtained on a Shimazu IR-408 spectrophotometer.

Measurement of Superoxide Dismutase like Activity. The superoxide dismutase like activity was examined by a method for the xanthine–xanthineoxidase system used by Fridovich et al.[17] The assay is performed in 3 mL of 0.05 M potassium phosphate buffer at pH 7.8 containing 10^{-4} M EDTA in a 1-cm cuvette thermostated at 25.0 °C. The reaction mixture contained 1×10^{-5} M ferricytochrome *c* (Sigma Chemicals Type-III), 1×10^{-4} M xanthine, 300 Sigma units of catalase (Sigma Chemicals, C-100), and sufficient xanthine oxidase (Sigma Chemicals) to produce a rate of reduction for ferricytochrome *c* (550 nm) of 0.025 absorbance unit/min. Under these conditions, the concentration of copper complexes required to halve the initial (till 5 min) rate is defined as IC_{50}. Since the Cu(I)–cimetidine complex is very insoluble in aqueous solution, the saturated solution was prepared and its concentration estimated from copper content by using an atomic absorption method with a Shimazu AA-646 flame spectrophotometer.

Results and Interpretation

Copper(II) Complex Formation Constants. The potentiometric titration curves for L·$HClO_4$ (L = cimetidine) and 1:1 and 1:11 Cu(II)–L·$HClO_4$ are displayed in parts a–c of Figure 1, respectively. The mixed protonation constant K_L defined by equation 1 was calculated to be $10^{7.20}$ at 25 °C and I = 0.20 M ($NaClO_4$),

$$L + H^+ \rightleftharpoons HL^+ \qquad K_L = [HL^+]/([L]a_{H^+}) \qquad (1)$$

which agrees well with the reported value of $10^{7.09}$ at 25 °C and I = 0.1 M (NaCl).[18] In the latter stage of the Cu(II)–cimetidine titrations the green 1:2 complex[5] started to precipitate; see the broken lines in Figure 1b,c. For equilibrium analysis, we have used the initial stage of the titration curve (part c) [$0.1 < a < 0.6$, where *a* is moles of base per moles of Cu(II) ion]. The data fit to simultaneous equilibria 2 and 3. The buffer pH region of

$$Cu^{II} + L \rightleftharpoons Cu^{II}\text{–}L \qquad K_{Cu^{II}L} = [Cu^{II}\text{–}L]/([Cu^{II}][L]) \qquad (2)$$

$$Cu^{II}\text{–}L + L \rightleftharpoons Cu^{II}\text{–}L_2 \qquad K_{Cu^{II}L_2} = [Cu^{II}\text{–}L_2]/([Cu^{II}\text{–}L][L]) \qquad (3)$$

part c is less than 5 and hence, the hydrolysis of Cu^{II}_{aq} is negligible in light of $K_{OH} = [Cu(OH)^+]/([Cu^{II}][OH^-]) = 10^{6.06}$ at 25 °C.[19] The calculated 1:1 and 1:2 Cu(II)–cimetidine formation constants $K_{Cu^{II}L}$ and $K_{Cu^{II}L_2}$ at 25 °C and I = 0.20 M ($NaClO_4$) are (3.02 ± 0.05) × 10^4 M^{-1} and (2.35 ± 0.05) × 10^4 M^{-1}, respectively.

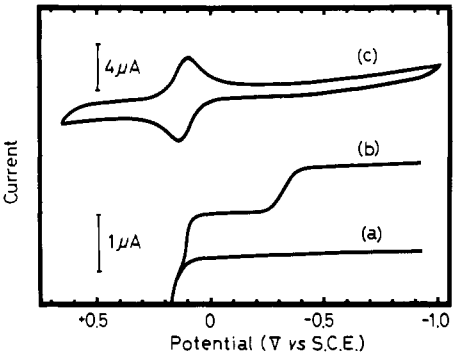


Figure 2. (a) Polarogram of 2.0×10^{-3} M cimetidine at pH 7.8, 25 °C and I = 0.20 M ($NaNO_3$). (b) Polarogram of 2.0×10^{-4} M 1:2 Cu(II)–cimetidine complex in the presence of 2.0×10^{-3} M cimetidine at pH 7.8, 25 °C and I = 0.20 M ($NaNO_3$). $E_{1/2}$ = +0.11, $E^2_{1/2}$ = −0.32 V vs. SCE. (c) Cyclic voltammogram of 2.0×10^{-4} M 1:2 Cu(II)–cimetidine complex in the presence of 2.0×10^{-3} M cimetidine at pH 7.8, 25 °C and I = 0.20 M ($NaClO_4$). $E'_{1/2}$ = +0.13 V vs. SCE.

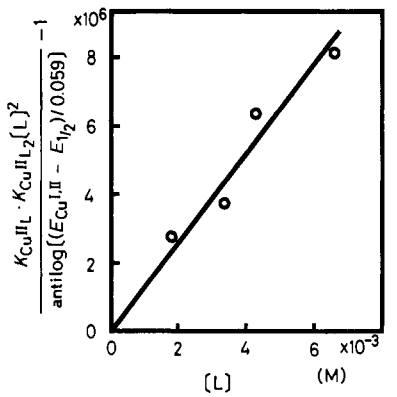


Figure 3. Plots of eq 9. $E_{1/2}$ values used for the calculation were obtained by using the polarographic method at pH 7.8, 25 °C, I = 0.20 M ($NaNO_3$), [total Cu(II)] = 2.0×10^{-4} M, and [total L] = 2.4×10^{-3} to 8.4×10^{-3} M.

Redox Properties. A typical polarogram of the 1:2 Cu(II)–cimetidine complex in aqueous solution at pH 7.8 (cimetidine was used 10 times in excess to suppress the cimetidine dissociation to the 1:1 complex) showed two-step reduction waves (Figure 2b). The first step at $E_{1/2}$ = +0.11 V vs. SCE represents a reversible one-electron-reduction process for the Cu(II) to the Cu(I) complex, which was checked by the log plot method.[20] The second reduction step at $E^2_{1/2}$ = −0.32 V is nonreversible, which represents the reaction of Cu(I) complex to form Cu^0–Hg. The cyclic voltammogram (Figure 2c) corresponding to the first polarographic wave indicates a reversible redox process on a glassy-carbon electrode. The midpoint potential $E'_{1/2}$ between the anodic and cathodic peak is +0.13 V vs. SCE. For a reversibility test, we have measured the cyclic voltammogram ($E'_{1/2}$ = +0.16 V vs. SCE) of Cu(II) with 5 equiv of cimetidine at pH 7.0 at the scan range of −0.20 to +0.50 V vs. SCE and scan rates of 10–100 mV s^{-1}, and proved that the cathodic and anodic peak separations are 58–65 mV, the ratio of the two peak heights is unity, and the peak heights are proportional to the square roots of the scan rates.

The $E_{1/2}$ values on the polarogram were found to shift to less positive potentials with an increase in ratio of [cimetidine] to [Cu^{II}]: +0.102 (12:1), +0.079 (22:1), +0.075 (29:1), and +0.064 (42:1) V vs. SCE at pH 7.8, 25.0 °C, and I = 0.20 M ($NaNO_3$). The lowering of the [cimetidine]:[Cu^{II}] ratio raises the $E_{1/2}$ value to ~+0.15 V vs. SCE (at 5:1) where the Hg oxidation wave merges. These $E_{1/2}$ values were used to calculate the theoretical Cu(I)–cimetidine complex formation constant K_{Cu^IL}.

Copper(I) Complex Formation Constants. The Cu(I)–cimetidine equilibria can be described by eq 4–6, which, along with

(17) McCord, J. M.; Fridovich, I. *J. Biol. Chem.* **1969**, *244*, 6049.
(18) Vochten, R.; Remaut, G.; Huybrechts, W. *J. Pharm. Pharmacol.* **1980**, *32*, 863.
(19) Davies, C. W. *J. Chem. Soc.* **1951**, 1256.
(20) Kolthoff, I. M.; Lingane, J. J. *Polarography*; Interscience: New York, 1952.

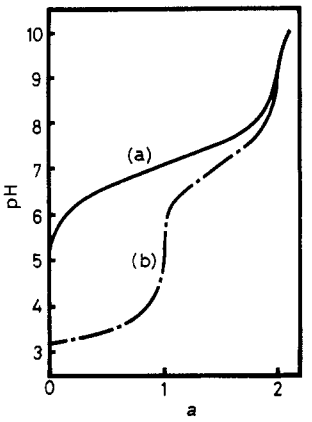


Figure 4. Potentiometric titration curves for L·HClO$_4$ (L = cimetidine) in the absence and presence of Cu(I) ion in 2% v/v CH_3CN at 25 °C and I = 0.2 M ($NaClO_4$): (a) [total L] = 2.0×10^{-3} M only; (b) [total Cu(I)] = 1.0×10^{-3} M, [total L] = 2.0×10^{-3} M. a is mole of base per mole of Cu(I) ion.

eq 2 and 3, are incorporated into the Nernst equation to obtain general formula in eq 7. Equation 7 can be simplified to eq 8

$$Cu^{I} + L \rightleftharpoons Cu^{I}\text{–}L \qquad K_{Cu^{I}L} \tag{4}$$

$$Cu^{I}\text{–}L + L \rightleftharpoons Cu^{I}\text{–}L_2 \qquad K_{Cu^{I}L_2} \tag{5}$$

$$Cu^{I}\text{–}L_{n-1} + L \rightleftharpoons Cu^{I}\text{–}L_n \qquad K_{Cu^{I}L_n} \tag{6}$$

$$E_{1/2} = E_{Cu^{I/II}} + \frac{RT}{F} \ln \left\{ \frac{1 + \sum_{j=1}^{n} \prod_{i=1}^{j} (K_{Cu^{I}L_i}[L])}{1 + K_{Cu^{II}L}[L] + K_{Cu^{II}L}K_{Cu^{II}L_2}[L]^2} \right\} \tag{7}$$

$$E_{1/2} = E_{Cu^{I/II}} + 0.059 \log \left\{ \frac{1 + \sum_{j=1}^{n} \prod_{i=1}^{j} (K_{Cu^{I}L_i}[L])}{K_{Cu^{II}L}K_{Cu^{II}L_2}[L]^2} \right\} \tag{8}$$

at [cimetidine] >> [Cu^{II}], pH >6, and 25 °C, since the Cu(II) complex is almost all in the 1:2 form. Rearrangement of eq 8 gives eq 9. With a displacement of −0.079 V vs. SCE for the

$$\frac{K_{Cu^{II}L}K_{Cu^{II}L_2}[L]^2}{\text{antilog } [(E_{Cu^{I/II}} - E_{1/2})/0.059]} - 1 =$$

$$K_{Cu^{I}L}[L]\{1 + \sum_{j=2}^{n} \prod_{i=2}^{j} (K_{Cu^{I}L_j}[L])\} = K_{Cu^{I}L}[L]\{1 + A([L])\} \tag{9}$$

$E_{Cu^{I/II}}$ term[20] and the above values for $K_{Cu^{II}L}$ and $K_{Cu^{II}L_2}$, a plot of the left-hand side against [L] of eq 9 gives a linear line with zero intercept (Figure 3), which indicates the $A([L])$ term to be negligibly small. That is, the 1:2 (or more) Cu^{I}–cimetidine complexes are virtually negligible under the reaction conditions. From the gradient of the linear line, we can obtain $K_{Cu^{I}L}$ = (1.3 ± 0.2) × 10^9 M^{-1}.

To get support for this conclusion, we have examined the direct interaction of Cu(I) ion with cimetidine (1:2 molar ratio) by a potentiometric acid–base titration method (Figure 4). When $Cu^{I}[NCCH_3]_4 \cdot ClO_4$ is mixed with L·HClO$_4$ in aqueous solution, the colorless 1:1 Cu(I)–cimetidine complex immediately and nearly quantitatively precipitated and at the same time 1 equiv of H^+ was liberated (Figure 4b). Hence the titration curve up to a = 1 overlaps with the titration curve of 1 equiv of H^+ in the presence of 1 equiv of L·H^+. The break at a = 1 (where all the liberated H^+ is completely neutralized) and the subsequent buffer region up to a = 2 [where the uncomplexed 1 equiv of L·H^+ (pK_a 7.20) is neutralized] well illustrate the 1:1 Cu(I)–cimetidine equilibrium of eq 4. The complex formation constant $K_{Cu^{I}L}$ for Cu(I) is apparently larger than $K_{Cu^{II}L}$ for Cu(II), as intuitively understood from the greater depression of the cimetidine buffer pH with Cu(I) (Figure 4b) than with Cu(II) (Figure 1b). Because of the precipitation problem, we were unable to evaluate the $K_{Cu^{I}L}$ value from the Figure 4b titration data.

The theoretical Nernst equation (10) for the 1:1 complex formation constants $K_{Cu^{I}L}$ and $K_{Cu^{II}L}$ allows us to assess a theoretical redox potential $E°$(1:1) of +0.18 V vs. SCE (at 25 °C) for the 1:1 Cu(I/II)–cimetidine complexes.

$$E°(1{:}1) = E_{Cu^{I/II}} + 0.059 \log \left(\frac{K_{Cu^{I}L}}{K_{Cu^{II}L}} \right) \tag{10}$$

Isolation and Characterization of the 1:1 Cu(I)–Cimetidine Complex. The $Cu^{I/II}$ redox potential (+0.18 V vs. SCE or +0.42 V vs. NHE) suggests appreciable stability of the Cu(I)–cimetidine complex with respect to the Cu(II) complex. In fact, we have succeeded in isolating an analytically pure 1:1 Cu(I)–cimetidine complex [Cu(L)(ClO_4)·H_2O] (colorless) by treating $Cu^{I}[NCCH_3]_4(ClO_4)$[15] with 2 equiv of cimetidine. The same species precipitated when the 1:2 Cu(II)-cimetidine complex (green, water-soluble) was chemically reduced with ascorbic acid or 0 V vs. SCE at pH 7 and 25 °C was electrochemically applied. This is the first report of isolation of a stable Cu(I)–cimetidine complex. The nitrile absorption in the IR spectrum of the 1:1 Cu(I)–cimetidine complex occurs as a strong singlet band at 2230 cm^{-1}, 30 cm^{-1} higher than that of the 1:2 Cu(II)–cimetidine complex.[5] The insufficient solubility of the cuprous complex in any solvent tested precluded its extensive characterization. Its maximum solubility in 0.05 M phosphate buffer at pH 7.8 and 25 °C was estimated to be 3.4×10^{-5} M by the atomic absorption spectroscopic method. The colorless cuprous complex is gradually oxidized in air to the green cupric complex at room temperature.

Superoxide Dismutase like Activity of Cu(I)- and Cu(II)–Cimetidine Complexes. The IC_{50} of the 1:1 Cu(I)–cimetidine complex was 4×10^{-7} M with an additional presence of 2.0×10^{-5} M cimetidine at 25 °C and pH 7.8. Separately, we have confirmed that free cimetidine has no SOD-like activity. The IC_{50} of the 1:2 Cu(II)–cimetidine complex was 4×10^{-6} M with the presence of 4.0×10^{-5} M cimetidine at 25 °C and pH 7.8. In order to assess those values, we have measured IC_{50} of previously reported SOD-like complexes under the same conditions: e.g. Cu^{II}[*o*-phen]$_2$,[21] 2×10^{-4} M; Cu(II)–glycylglycine,[22] 2×10^{-5} M; Cu^{II}[salicylate]$_2$,[23] 4×10^{-4} M; Cu(II)–macrocyclic dioxo[12]N_4,[22] 5×10^{-4} M.

Discussion

Affinity of Cimetidine for Cu(II). Analysis of the pH-metric titration curve c in Figures 1 before precipitation of the 1:2 Cu(II)–cimetidine complex has established the complexing equilibria of 1:1 (eq 2) and 1:2 (eq 3) in aqueous solution with formation constants $K_{Cu^{II}L}$ = (3.02 ± 0.05) × 10^4 M^{-1} and $K_{Cu^{II}L_2}$ = (2.35 ± 0.05) × 10^4 M^{-1}. As in an earlier report,[5] only the very insoluble (polymeric) 1:2 cupric complex was isolated from a mixture of $Cu^{II}(ClO_4)_2$ and cimetidine in 1:1 to 1:11 molar ratios. We were unable to isolate the 1:1 complex. The coordination of cimetidine to Cu(II) in the 1:2 complex in solution will occur through the two imidazoles and two thioethers at equatorial site and weakly bound two cyanoguanidine (or H_2O) at axial positions (in crystals the cyano-N goes to an axial site of an adjacent molecule). For the 1:1 complex the three donors from a cimetidine may occupy three equatorial sites.

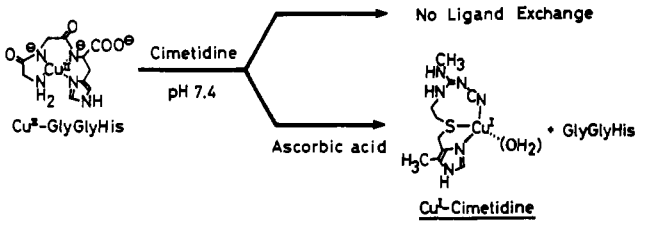
Apparently the cimetidine binding to Cu(II) ion is not so strong as that of the relevant physiological bidentate histamine ($K_{Cu^{II}L}$ = 3.8×10^9 M^{-1}, $K_{Cu^{II}L_2}$ = 3.0×10^6 M^{-1}),[24] histidine ($K_{Cu^{II}L}$ = 2.0×10^{10} M^{-1}, $K_{Cu^{II}L_2}$ = 4.2×10^7 M^{-1}),[24] or other amino acids (e.g., with glycine, $K_{Cu^{II}L}$ = 2.4×10^8 M^{-1}, $K_{Cu^{II}L_2}$ = 7.4×10^6 M^{-1}).[25] Cimetidine–Cu is less stable than tetradentate glycyl-

(21) Goldstein, S.; Czapski, G. *J. Am. Chem. Soc.* **1983**, *105*, 7276.
(22) Kimura, E.; Sakonaka, A.; Nakamoto, M. *Biochem. Biophys. Acta* **1981**, *678*, 172.
(23) de Alvare, L. R.; Goda, K.; Kimura, T. *Biochem. Biophys. Res. Commun.* **1976**, *69*, 687.
(24) Sovago, I.; Kiss, T.; Gergely, A. *J. Chem. Soc., Dalton Trans.* **1978**, 964.

glycylhistidine (GGH)–Cu that is a biomimetic model of Cu(II) binding to albumin,[26,27] whose 1:1 complex formation constant at pH 7.4 is calculated to be 6.3×10^{12} M^{-1},[26] approximately 10^8 times as great as the $K_{Cu^{II}L}$ value for the cimetidine complex. This argument is supported by a qualitative test; addition of a 10 times excess of cimetidine to the Cu(II)–GGH complex (λ_{max} = 525 nm) at pH 7.4 causes no visible absorption spectral change to the 1:2 Cu(II)–cimetidine (λ_{max} = 606 nm), indicating no occurrence of the ligand exchange. These in vitro experiments taken together can exclude an earlier conception[5] that cimetidine might be bound with Cu(II) in our body.

Redox Potential of Cu(I/II)–Cimetidine. The theoretical redox potential of the 1:1 Cu(I/II)–cimetidine complex is extremely high, +0.42 V vs. NHE at pH 7.8. The presence of higher concentration of cimetidine with respect to [Cu^{II}] has lowered the potential to +0.31 V vs. NHE at 42:1. This is interpreted to mean that the higher [cimetidine] makes the six-coordinate 1:2 complex more favorable, which contributes to stabilization of Cu(II).

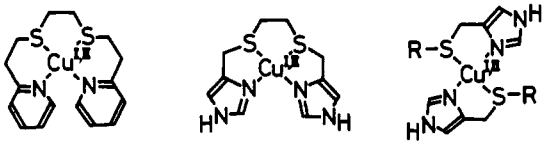
The equilibrium mixtures of 1:1 and 1:2 Cu(II)–cimetidine complexes at pH 7.0 were readily and quantitatively reduced with ascorbic acid ($E°$ = +0.22 V vs. NHE at pH 7)[28] and dithiothreitol ($E°$ = −0.33 V vs. NHE at pH 7)[29] to the colorless 1:1 Cu(I)–cimetidine complex. The thus formed Cu(I) complex solution shows an cyclic voltammogram identical with the one obtained initially with Cu(II) complex ($E'_{1/2}$ = +0.40 V vs. NHE, at pH 7.0 and [cimetidine]/[Cu^{II}] = 5).



Most interestingly from a physiological point of view, Cu(II) tightly bound to GGH at pH 7.4 is rapidly released to bind with cimetidine in the presence of ascorbic acid (10 mM) (the half-life time is ca. 5 min, when [Cu^{II}–GGH] = 1 mM and [cimetidine] = 2 mM), giving the insoluble 1:1 Cu(I)–cimetidine complex. In our body, there are other biological reductants that possess less positive $E°$ values than $Cu^{II/I}$–cimetidine; e.g. hemoglobin (+0.17 V vs. NHE at pH 7), ubiquinone (+0.10 V at pH 7), and glutathione (−0.23 V at pH 7).[28] These facts may suggest that the administered cimetidine can interact with available copper ions and that the resulting Cu(I)–cimetidine species precipitates on tissues. The present in vitro discovery might further be relevant to a pharmacological fact that cimetidine binding to "imidazole" receptors is greatly enhanced in the presence of Cu(II) ion and ascorbic acid.[9] Physiologically of further interest may be a fact that the Cu(I)–cimetidine complex is stable even at very low pH region, as illustrated in Figure 4b, which suggests that cimetidine could stay bound to the cuprous complex in digestive organs. In this connection our preliminary test has revealed that the cimetidine activity inhibiting the acid secretion in rats is dramatically enhanced by addition of copper ion. We are further pursuing experiments for the effects of copper ion on the cimetidine activities.

Cimetidine as a Biomimetic Ligand for Interconversion of Cu(I/II). The 1:1 Cu(L)–cimetidine complex formation constant $K_{Cu^{I}L}$ = 1.3×10^9 M^{-1}, which is greater than that for the 1:1 Cu(II)–cimetidine complex, is remarkably large for a Cu(I) complex in aqueous solution. We do not know of such a thermodynamically stable Cu(I) complex in aqueous solution with any of the previously reported ligands except for macrocyclic tetrathioethers.[30] All of the three donor groups of cimetidine, i.e. imidazole N, thioether S, and cyanoguanidine N, have π-donor characters and hence would prefer the soft Cu(I) ion over the hard Cu(II) ion. The IR spectrum of the Cu(I)–cimetidine solid shows a strong $\nu_{C\equiv N}$ at 2230 cm^{-1}, 30 cm^{-1} higher than that for $Cu^{II}L_2$, suggesting a stronger –CN–Cu(I) bonding. Uncomplexed cimetidine has $\nu_{C\equiv N}$ at 2180 cm^{-1}. In the tetrahedral [Cu^{I}(NCCH_3)$_4$]ClO_4 complex, $\nu_{C\equiv N}$ occurs at 2275 and 2300 cm^{-1}.[14] The unique electrochemical properties of cimetidine which favor the Cu(I) ion are obvious when its $E°$ value is compared with those of relevant ligand systems: tetraimidazole, $E°$ = +0.04 V vs. NHE;[31] tetraammine $E°$ = +0.02 V vs. NHE;[31] bis(*o*-phenanthroline), $E°$ = +0.17 V vs. NHE.[32]

Recently, copper complexes containing thioether and heteroaromatic nitrogen ligands are attracting much attention as models[33,34] for type I copper proteins ($E°$ = +0.2 to +1.0 V vs. NHE[10]) having electron-transfer functions. Typical model structures are shown below. Their reported $E°$ values (vs. NHE) are +0.9 V (for **1** in H_2O),[33] +0.47 V (for **2** in MeOH),[34] and



1[33] **2**[34] **3**[34]

+0.61 V (for **3** with R = *t*-Bu in MeOH).[34] Cimetidine has a similar structure to those, especially **3** with an additional cyanoguanidine. The electronic absorption bands of the possible square-planar (or octahedral) $Cu^{II}L_2$ complex (λ_{max} = 344 and 615 nm in MeOH)[5] and of square-planar Cu(II) in **3** (R = *t*-Bu; λ = 340 and 624 nm in MeOH/EtOH)[33] are very close, indicating similar ligand fields for cimetidine and **3**. The more intense bands at ~340 nm for both are assigned to S → Cu charge-transfer transitions.[5,34]

Superoxide Dismutase like Activity by Cu–Cimetidine Complexes. The $E°$ value of +0.42 V vs. NHE for Cu(I/II)–cimetidine is especially interesting in its similarity to the reported $E°$ value of +0.42[11] or +0.35 V[12] vs. NHE (at pH 7) for Cu–superoxide dismutase (Zn·Cu–SOD). We therefore have assayed the SOD-like activity of the Cu(I/II)–cimetidine complexes to compare with the previous, SOD-like complexes by the well-established xanthine–xanthine oxidase and ferricytochrome *c* system.[17] In our earlier experiments,[35] we have found parallel O_2^- scavenging activities of indirectly (from xanthine–xanthine oxidase) and directly (from KO_2) generated O_2^- for various copper complexes.

The assay result was very encouraging in that the 1:1 Cu(I)– and 1:2 Cu(II)–cimetidine complexes are extremely strong O_2^- scavengers, whose activities are much higher than any of the previously recognized SOD-like substances: Cu(II)–(*o*-phen)$_2$,[21] Cu(II)–glycylglycine,[22] Cu(II)–(salicylate),[23] and Cu(II)–macrocyclic polyamines.[35] Although the detailed reaction mechanism must await more precise experiments, we tentatively consider the O_2^- dismutation reactions 11 and 12 to be similar

$$\text{Cu(II)–cimetidine} + O_2^- \rightarrow \text{Cu(I)–cimetidine} + O_2 \quad (11)$$

$$\text{Cu(I)–cimetidine} + O_2^- + 2H^+ \rightarrow \text{Cu(II)–cimetidine} + H_2O_2 \quad (12)$$

to those with SOD, in view of nearly the same $E°$ values for $Cu^{I/II}$. The active species most likely is the 1:1 Cu–cimetidine species (the 1:2 Cu(II)–cimetidine complex should undergo dissociation

(25) Basolo, F.; Chen, Y. T. *J. Am. Chem. Soc.* **1954**, *76*, 953.
(26) Lau, S.-J.; Kruck, T. P. A.; Sarkar, B. *J. Biol. Chem.* **1974**, *249*, 5878.
(27) Sakurai, T.; Nakahara, A. *Inorg. Chem.* **1980**, *19*, 847.
(28) Dryhurst, G.; Kadish, K. M.; Scheller, F.; Renneberg, R. *Biological Electrochemistry*; Academic: New York, 1982; Vol. 1.
(29) Windholz, M.; Budavari, S.; Stroumtsos, L. Y.; Fertig, M. N. *Merck Index*, 9th ed., Merck: Rahway, NJ, 1976; p 3394.
(30) Jones, T. E.; Rorabacher, D. B. *J. Am. Chem. Soc.* **1975**, *97*, 7485.
(31) Li, N. C.; White, J. M.; Doody, E. *J. Am. Chem. Soc.* **1954**, *76*, 6219.
(32) James, B. R.; Williams, R. J. P. *J. Chem. Soc.* **1961**, 2007.
(33) Brubaker, G. R.; Brown, J. N.; Yoo, M. K.; Kinsey, R. A.; Kutchan, T. M.; Mottel, E. A. *Inorg. Chem.* **1979**, *18*, 299.
(34) Aoi, N.; Matsubayashi, G.; Tanaka, T. *J. Chem. Soc., Dalton Trans.* **1983**, 1059.
(35) Kimura, E.; Yatsunami, A.; Watanabe, A.; Machida, R.; Koike, T.; Fujioka, H.; Kuramoto, Y.; Sumomogi, M.; Kunimitu, K.; Yamashita, A. *Biochem. Biophys. Acta* **1983**, *745*, 37.

prior to the 1:1 species), whose distinct features may be (i) that cimetidine is a tridentate ligand and hence the remaining one coordination site is open to incoming O_2^-, (ii) that cimetidine is a neutral ligand and hence the complexes bear positive net charges, ready to invite the attack of negative superoxide ion O_2^-, and (iii) that the Cu(I) $\rightleftharpoons$ Cu(II) conversion is nearly reversible without decomposition of the complexes. In Cu–SOD,[36] the Cu(II) in the active center is surrounded by four imidazole nitrogens and one H_2O that is to be displaced by the incoming O_2^-. For further advantage Cu–SOD has Arg 141 (bearing a positive guanidinium cation, which helps to attract the negative O_2^- to the H_2O position.[37]

Recently, cimetidine has been reported to inhibit the oxidative metabolisms of steroid hormones,[38,39] drugs,[40–43] and other chemicals.[41] It was proposed that cimetidine directly binds at the sixth ligand position of cytochrome P-450.[44,45] Our present findings may invoke a new explanation that the active O_2 species formed on P-450 may be scavenged by the Cu–cimetidine complexes before they reach the substrates. We are planning to test this hypothesis. In this regard, it may be recalled that Cu(II) complexes of tyrosine, salicylates, etc. having SOD-like activities are also reported to inhibit drug metabolisms by microsomal cytochrome P-450.[46,47] In light of the recent report[48] that a Cu(II) complex of 3,5-diisopropylsalicylate exhibiting a strong SOD-like activity is promising as antiinflammatory and anticancer agents, the copper–cimetidine complexes are certainly worthy of thorough pharmacological investigations.

Registry No. $[CuL(H_2O)]ClO_4$, 102234-26-4.

(36) Tainer, J. A.; Getzoff, E. D.; Richardson, J. S.; Richardson, D. C. *Nature (London)* **1982**, *306*, 284.
(37) Getzoff, E. D.; Tainer, J. A.; Weiner, P. K.; Kollmann, P. A.; Richardson, J. S.; Richardson, D. C. *Nature (London)* **1983**, *306*, 287.
(38) Feely, J.; Robertson, D.; Island, D. P.; Wood, A. J. J. *N. Engl. J. Med.* **1982**, *306*, 1054.
(39) Morita, K.; Ono, T.; Shimakawa, H.; Wada, F. *Chem. Pharm. Bull.* **1984**, *32*, 4043.
(40) Knodell, R. G.; Holtzmann, J. L.; Crankshaw, D. L.; Stelle, N. M.; Stanley, L. N. *Gastroenterology* **1982**, *82*, 84.
(41) Pelkonen, O.; Puurunen, J. *Biochem. Pharmacol.* **1980**, *29*, 3075.
(42) Klotz, U.; Reimann, I. *N. Engl. J. Med.* **1980**, *302*, 1012.
(43) Desmond, P. V.; Patwardhan, R.; Parker, R.; Schenker, S.; Speeg, K. V. *Life Sci.* **1980**, *26*, 1261.
(44) Wilkinson, C. F.; Hetnarski, K.; Yellin, T. O. *Biochem. Pharmacol.* **1972**, *21*, 3187.
(45) Rendic, S.; Sunjic, V.; Toso, R.; Kajfez, F.; Ruf, H. *Xenobiotica* **1979**, *9*, 555.
(46) Richter, C.; Azzi, A.; Weser, U.; Wendel, A. *J. Biol. Chem.* **1977**, *252*, 5061.
(47) Werringloer, J.; Kawano, S.; Chacos, N.; Estabrook, R. W. *J. Biol. Chem.* **1979**, *254*, 11839.
(48) Sorenson, J. R. J. *Chem. Br.* **1984**, 1110.

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Electrochemistry of $[M(CO)_3Cp]_2$, $[M(CO)_3Cp]^+$, $[M(CO)_3Cp]^-$, and $M(CO)_3Cp$ Where M = Mo and W

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The complete electrochemical mechanisms for the reduction and oxidation of $[Mo(CO)_3Cp]_2$ and $[W(CO)_3Cp]_2$, where Cp is cyclopentadienyl, and their associated monomeric cationic and anionic species were determined by the use of various electrochemical techniques. The dimers are both reduced and oxidized by an overall two-electron ECE mechanism, where the chemical reaction produces the monomeric 17-electron $M(CO)_3Cp$ radical (M = Mo, W). This radical is a key intermediate species in the electrochemical mechanism. It was demonstrated that $[Mo(CO)_3Cp]^+$ and $[M(CO)_3Cp]^-$ could be directly interconverted by a two-electron-transfer step. This latter reaction proceeds through the monomeric $M(CO)_3Cp$ radical species.

Introduction

The heterometallic dimer (TPP)$InMo(CO)_3Cp$ and (TPP)-$InW(CO)_3Cp$, where TPP is the dianion of tetraphenylporphyrin,[1,2] may be oxidized or reduced by one or more electrons and, depending upon the potential, will generate as decomposition products either $[M(CO)_3Cp]_2$, $[M(CO)_3Cp]^+$, $[M(CO)_3Cp]^-$, or $M(CO)_3Cp$ where M = Mo or W.[2] In this regard, it is of some importance to understand the detailed electrochemistry of the molybdenum and tungsten complexes in the absence of a metalloporphyrin. This is the subject of the present communication.

The electrochemistry of Mo and W metal–metal-bonded complexes of the form $[M(CO)_3Cp]_2$ has been the subject of only several brief studies.[3–6] In nonaqueous media, the addition of two electrons to $[M(CO)_3Cp]_2$ generates the anionic $[M(CO)_3Cp]^-$ fragment, which is formed after rapid cleavage of the metal–metal bond. The abstraction of two electrons from $[M(CO)_3Cp]_2$ also results in rapid cleavage of the metal–metal bond, this time resulting in formation of $[M(CO)_3Cp]^+$. However, a complete electrochemical mechanism for these oxidations and reductions has never been reported. For example, the overall two-electron reduction of $[M(CO)_3Cp]_2$ can generate two $[M(CO)_3Cp]^-$ anions either by a single two-electron reduction, or by two one-electron reductions both of which are followed by cleavage of the metal–metal bond (an electrochemical EC or an EEC mechanism). Alternatively, the reduction may proceed first via the singly reduced $[M(CO)_3Cp]_2^-$ radical and then via the $M(CO)_3Cp$ fragment that is formed upon cleavage of the metal–metal bond in $[M(CO)_3Cp]_2^-$ (an electrochemical ECE mechanism).

The photolysis of $[M(CO)_3Cp]_2$ and $[W(CO)_3Cp]_2$ leads to homolytic cleavage of the metal–metal bond as shown in eq 1

$$[M(CO)_3Cp]_2 \xrightarrow{h\nu} 2M(CO)_3Cp \qquad (1)$$

where M = Mo[7,8] or W.[7,9] The $M(CO)_3Cp$ radical has also been postulated as an intermediate during electroreduction of the

(1) Cocolios, P.; Chang, D.; Vittori, P.; Guilard, R.; Moise, C.; Kadish, K. M. *J. Am. Chem. Soc.* **1984**, *106*, 5724.
(2) Kadish, K. M.; Guilard, R., manuscript in preparation.
(3) Madach, T.; Vahrenkamp, H. *Z. Naturforsch., B.: Anorg. Chem., Org. Chem.* **1979**, *34B*, 573.
(4) Dessy, R. E.; Stary, F. E.; King, R. B.; Waldrop, M. *J. Am. Chem. Soc.* **1966**, *88*, 471.
(5) Dessy, R. E.; Weissman, P. M.; Pohl, R. L. *J. Am. Chem. Soc.* **1966**, *88*, 5117.
(6) Denisovitch, L. I.; Gubin, S. P.; Chapovskii, Y. A.; Ustynok, N. A. *Bull. Acad. Sci. USSR, Div. Chem. Sci. (Engl. Transl.)* **1971**, *20*, 1851.
(7) Wrighton, M. S.; Ginley, D. S. *J. Am. Chem. Soc.* **1975**, *97*, 4246.
(8) Hughey, J. L.; Bock, C. R.; Meyer, T. J. *J. Am. Chem. Soc.* **1975**, *97*, 4440.
(9) Laine, R. M.; Ford, P. C. *Inorg. Chem.* **1977**, *16*, 388.

0020-1669/86/1325-2246$01.50/0