to explain our kinetic and CD data.

Conclusions about the Comparison of ApG and GpA. From the comparison of the platinum-binding reactions one can draw the following conclusions: (i) ApG and GpA reacting with *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ first give GN7 platination to an intermediate complex, followed by a chelation step. (ii) For the first platination step, ApG reacts 2 times faster than GpA due to the influence of the phosphodiester group. (iii) The chelation step of the ApG intermediate complex **8** is 3 times slower than that of the GpA intermediate. This can be attributed to a stabilizing hydrogen bond between a ligand of the GN7-bound cationic platinum species and the anionic phosphodiester group. (iv) This interaction in complex **8** precludes guanine rotation about the glycosidic bond and favors the stacking of the bases, leading practically to the single AN7,GN7 anti,anti ApG·*cis*-Pt chelate **1**. (v) There is no interaction within the GpA intermediate complex to limit the conformational freedom of the bases. Therefore, adenine chelation can occur through the N1 and N7 binding sites, with anti and syn conformations of the bases, leading to one GN7,AN1 (**2**) and three GN7,AN7 (**3–5**) GpA·*cis*-Pt isomeric chelates. (vi) Several of the platinum chelations observed with GpA are not relevant to the case of DNA platination, especially the GN7,AN1 one. But our dinucleotide results show that there is no intrinsic property of the ApG or GpA sequence to explain the absence of d(GpA) adduct in platinated DNA. It is noteworthy that for d(GpApG), even at 80 °C, no GpA chelate is found and the G5′ intermediate complex is only chelated by 3′-GN7.[51] However, this seems mainly due to the greater reactivity of G vs. that of A[50] because, at 80 °C, the conformational mobility of the trinucleotide should enable the adenine binding sites to reach the platinum linked to 5′-GN7 (see GpA), as does the more remote 3′-GN7. Therefore, in the case of a stacked B-DNA type structure, if the first platinated G belongs to a AG or GA sequence, the formation of d(ApG) but no d(GpA) chelate is likely to result from the smaller distance between the platinum and N7 of 5′-A (≃3 Å) than between platinum and N7 of 3′-A (≃5 Å), as previously proposed by Dewan.[11] Within the GN7-coordinated intermediate complex, hydrogen bonding between the 5′-phosphodiester bridge and an ammine or aqua ligand, suggested by our ApG results, should increase the proximity, favoring the chelation by the 5′-neighboring base.

(56) We have checked that complex **8** is unchanged under the HPLC conditions (10^{-2} M $CH_3CO_2NH_4$, pH 4.7) by comparing its retention time with that of the corresponding acetato intermediate prepared by treatment of **8** with 0.2 M $CH_3CO_2NH_4$. HPLC elution with 10^{-2} M NH_4NO_3, pH 4.7, was also used.

Acknowledgments. Support of this work by a grant from the "Association pour la Recherche sur le Cancer" is gratefully acknowledged. We thank Drs. J. Y. Lallemand and E. Guittet for the 400-MHz ^1H NMR spectra and fruitful discussions and Dr. Gouyette for the atomic absorption determinations. Stimulating discussions with the group of Prof. Dr. J. Reedijk were made possible thanks to the sponsorship of the French-Dutch cultural agreement and the Direction de la Coopération et des Relations Internationales. We thank Engelhardt Industries, France, for a loan of platinum salt.

Contribution from the Dipartimento di Scienze Chimiche, Università di Catania, 95125 Catania, Italy, and Department of Chemistry, Brigham Young University, Provo, Utah 84602

Coordination Properties of Cyclopeptides. Formation, Stability, and Structure of Proton and Copper(II) Complexes of *cyclo*-(L-Histidyl-L-histidyl) in Aqueous Solution

Giuseppe Arena,*[1a] Raffaele P. Bonomo,[1a] Giuseppe Impellizzeri,[1a] Reed M. Izatt,*[1b] John D. Lamb,[1b] and Enrico Rizzarelli*[1a]

Received May 23, 1986

Cyclic dipeptides with amino acid residues containing complexing side-chain groups can coordinate to metal ions in a way that mimics the coordination sites in enzymes. In order to obtain an improved understanding of the complexing properties of cyclopeptides involved in biological systems, the interactions in aqueous solution of protons and copper(II) with *cyclo*-(L-histidyl-L-histidyl) (Cyhis) were studied by potentiometric pH titrations and by calorimetry. Values of $\Delta H°$ and $\Delta S°$ for proton interaction with Cyhis suggest that solvophobic forces are involved in the stabilization of the Cyhis folded conformation. Comparison between the enthalpy and entropy changes associated with Cu–Cyhis interaction and those associated with the interaction of Cu^{2+} with imidazole (Im) helps to identify the donor atoms involved in the coordination. The EPR data, namely the superhyperfine pattern arising from the interaction of the odd electron with the nitrogen atoms, confirm the suggestions advanced on the basis of thermodynamic values involving the formation of chelates of unusual ring size for both $[Cu(Cyhis)]^{2+}$ and $[Cu(Cyhis)_2]^{2+}$. In addition, the EPR spectra support the coordination of both nitrogens of the Im residues in the Cu(Cyhis) species. Solvophobic forces have been invoked to explain the thermodynamic values associated with $[Cu_2(Cyhis)_2H_{-2}]^{2+}$ complex formation.

Introduction

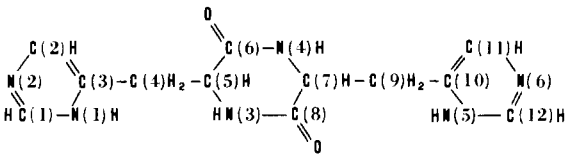
Naturally occurring and synthetic cyclic peptides have been the subject of intensive study in recent years[2-6] in modeling protein conformation, membrane transport, and other biological processes associated with ion binding.[7-10] The advantages of these model ligands over linear peptides are their "constrained" geometry and the absence in them of free $-COO^-$ and $-NH_2$ terminal groups. Unlike the case for linear peptides, the deprotonation of the peptide nitrogen occurs in the presence of transition-metal ions at high pH values[11] and therefore, under normal conditions, the macrocyclic peptides coordinate via oxygen atoms only.

Cyclic peptides with amino acid residues containing complexing side-chain substituents such as imidazole (Im), carboxylate, or thioether groups can coordinate to metal ions in a way that mimics the coordination sites in enzymes.[12] In addition, such chains can "encourage" the coordination of peptide nitrogens.[13] Recent studies involving cyclic peptides have focused on the synthesis of their metal complexes and the subsequent characterization of these

(1) (a) Università di Catania. (b) Brigham Young University.
(2) Ovchinnikov, Yu. A.; Ivanov, V. T. *Tetrahedron* **1975**, *31*, 2177.
(3) Deber, D. M.; Madison, V.; Blout, E. R. *Acc. Chem. Res.* **1976**, *9*, 106.
(4) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512.
(5) Kopple, K. *J. Pharm. Sci.* **1972**, *61*, 1345.
(6) Madison, V.; Atreyl, M.; Deber, C. M.; Blout, E. R. *J. Am. Chem. Soc.* **1974**, *96*, 6725.
(7) Blout, E. R.; Deber, C. M.; Pease, L. G. In *Peptides, Polypeptides and Proteins*; Blout, E. R., Bovey, F. A., Goodman, M., Lotan, N., Eds.; Wiley: New York, 1974; p 266.
(8) Sarkar, B. In *Metal Ligand Interaction in Organic Chemistry and Biochemistry*; Pullman, B., Boldblun, N., Eds.; Reidel: New York, 1977; Part 1, p 193.
(9) Baron, D.; Pease, L. G.; Blout, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 8299.
(10) Pease, L. G.; Watson, C. J. *J. Am. Chem. Soc.* **1978**, *100*, 1279.
(11) Rybka, J. S.; Margerum, D. W. *Inorg. Chem.* **1980**, *19*, 2784.
(12) Isied, S. S.; Kuehn, C. G.; Lyon, J. M.; Merrifield, R. B. *J. Am. Chem. Soc.* **1982**, *104*, 2632.
(13) Brubaker, G. R.; Sakkab, N. Y. *Bioinorg. Chem.* **1974**, *3*, 243.

0020-1669/87/1326-0795$01.50/0

complexes in the solid state.[13-27] While characterization of the complexes in solution is difficult, thermodynamic studies can be revealing.[28-32] In the present study, the thermodynamic parameters relating to the interaction of protons and copper(II) with the cyclic dipeptide *cyclo*-(L-histidyl-L-histidyl) (Cyhis) (I) have



I

been determined. This system was chosen because (i) histidine-containing cyclic dipeptides play an important role in biological systems, (ii) copper(II) enhances the catalytic action of histidine containing peptides,[33,34] and (iii) Cu(II)–Im bonding either has been demonstrated or has been inferred for proteins.[35-42] Few data have been published for interaction of transition-metal ions with cyclopeptides,[11,15,18,43] and to our knowledge, no $\Delta H°$ or $\Delta S°$ values have been reported. To aid in the interpretation of the data, a calorimetric study of the interaction of protons and copper(II) with Im and an EPR study of copper(II)–Cyhis interaction at different pH values have been carried out.

Table I. Thermodynamic Parameters for the Interactions of H^+ with Im and Bim and of Im with Cu^{2+} at 25 °C and Ionic Strength 0.1 mol dm^{-3}

reacn	log K	$\Delta H°$,[a] kcal mol^{-1}	$\Delta S°$,[a] cal mol^{-1} deg^{-1}
$H^+ + Im = [ImH]^+$	7.01[b]	−8.83 (4)	2.4 (1)
$H^+ + Bim = [BimH]^+$	7.39[c]		
$H^+ + [BimH]^+ = [BimH_2]^{2+}$	5.61[c]		
$Cu^{2+} + Im = [Cu(Im)]^{2+}$	4.33[b]	−6.94 (7)	−3.5 (2)
$Cu^{2+} + 2Im = [Cu(Im)_2]^{2+}$	7.61[b]	−13.4 (1)	−9.9 (5)
$Cu^{2+} + 3Im = [Cu(Im)_3]^{2+}$	10.31[b]	−20.1 (2)	−20.2 (8)
$Cu^{2+} + 4Im = [Cu(Im)_4]^{2+}$	12.2[b]	−23.6 (8)	−23 (3)

[a] Uncertainties given in parentheses as 3σ. [b] Reference 52. [c] Reference 43.

Table II. Thermodynamic Parameters for the Interaction of H^+ with Cyhis and of Cyhis with Cu^{2+} at 25 °C and Ionic Strength 0.1 mol dm^{-3}

reacn	log K	$\Delta H°$,[a] kcal mol^{-1}	$\Delta S°$,[a] cal mol^{-1} deg^{-1}
$H^+ + Cyhis = [CyhisH]^+$	6.531 (3)	−7.31 (1)	5.35 (3)
$H^+ + [CyhisH]^+ = [CyhisH_2]^{2+}$	5.491 (3)	−6.78 (9)	2.0 (3)
$Cu^{2+} + Cyhis = [Cu(Cyhis)]^{2+}$	6.01 (2) (6.10)[b]	−9.88 (7)	−5.7 (2)
$[Cu(Cyhis)]^{2+} + Cyhis = [Cu(Cyhis)_2]^{2+}$	4.55 (2) (4.88)[b]	−7.1 (1)	−2.9 (4)
$2Cu^{2+} + 2Cyhis = [Cu_2(Cyhis)H_{-2}] + 2H^+$	3.46 (3)	−4.3 (1)	1.5 (5)

[a] Uncertainties given in parentheses as 3σ. [b] Reference 15.

Experimental Section

Copper(II) nitrate was prepared from copper(II) basic carbonate by adding a slight excess of HNO_3. The concentrations of stock solutions were determined by EDTA titrations with murexide as the indicator. The excess HNO_3 was determined by Gran's method and by the ACBA computer program (see Calculations). Stock solutions of HNO_3 and KOH were determined by titration with primary standard tris(hydroxymethyl)aminomethane (THAM) and potassium hydrogen phthalate, respectively. Potassium nitrate and Im were used without further purification. All solutions were prepared with doubly distilled water.

Ligand Synthesis. Cyhis was synthesized by cyclization of L-histidine methyl ester dihydrochloride in MeOH at 37 °C.[44] Recrystallization from water gave a microcrystalline product that after drying, had mp 299–300 °C dec and $[\alpha]^{20}_D = -60.5°$ (1 N HCl, *C* 1), in accordance with previous literature data.[44] The proton NMR spectra[45] were also in agreement with those reported previously[45] for Cyhis in D_2O at room temperature. We also carried out mass spectral experiments and found a molecular peak.

Potentiometric Titrations. Computer-controlled potentiometric titrations were performed with an Orion digital pH meter (Model 801A) equipped with EIL glass and Ingold saturated calomel electrodes. The titration cell was thermostated at 25.0 ± 0.2 °C, and all solutions were maintained under an atmosphere of nitrogen, which was bubbled through a solution of the same ionic strength and temperature as those of the

(14) Sarkar, B. *Prog. Macrocyclic Chem.* **1981**, *2*, 251.
(15) Kojima, Y. *Chem. Lett.* **1981**, 61.
(16) Iyer, D. S. M.; Laussac, J. P.; Lau, S.; Sarkar, B. *Int. J. Pept. Protein Res.* **1981**, *17*, 549.
(17) Iyer, K. S. N.; Laussac, J. P.; Sarkar, B. *Int. J. Pept. Protein Res.* **1981**, *18*, 468.
(18) Bressan, M.; Bon, M. L.; Marchiori, F. *Inorg. Chim. Acta* **1982**, *67*, L47.
(19) Kojima, Y.; Hirotsu, K.; Matsumoto, K. *Chem. Lett.* **1976**, 809.
(20) Kojima, Y.; Hirotsu, K.; Matsumoto, K. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 3222.
(21) Ettorre, R.; Guantieri, V.; Tamburro, A. M.; De Nardo, L. *Inorg. Chim. Acta* **1979**, *32*, L39.
(22) Hori, F.; Kojima, Y.; Matsumoto, K.; Ooi, S.; Kuroya, H. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1076.
(23) Bressan, M.; Ettorre, R.; Marchiori, F.; Valle, G. *Int. J. Pept. Protein Res.* **1982**, *19*, 402.
(24) Guantieri, V.; Pandolfo, L.; Tamburro, A. M. *Inorg. Chim. Acta* **1982**, *67*, L51.
(25) Kojima, Y.; Yamashita, T.; Ishino, Y.; Hiroshima, T.; Hirotsu, K. *Chem. Lett.* **1983**, 453.
(26) Kojima, Y.; Yamashita, T.; Ishino, Y.; Hiroshima, T.; Miwa, T. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 3841.
(27) Valle, G.; Ettorre, R. *J. Chem. Soc., Dalton Trans.* **1983**, 453.
(28) Arhland, S. *Struct. Bonding (Berlin)* **1973**, *15*, 167.
(29) Corrie, A. M.; Williams, D. R. *J. Chem. Soc., Dalton Trans.* **1976**, 1068.
(30) Lim, M. C.; Nancollas, G. H. *Inorg. Chem.* **1971**, *10*, 1957.
(31) Bonomo, R. P.; Cali, R.; Riggi, F.; Rizzarelli, E.; Sammartano, S.; Siracusa, G. *Inorg. Chem.* **1979**, *18*, 3417.
(32) Arena, G.; Cali, R.; Cucinotta, V.; Musumeci, S.; Rizzarelli, E.; Sammartano, S. *J. Chem. Soc., Dalton Trans.* **1984**, 1651.
(33) Losse, G.; Barth, A.; Lagenbeek, W. *Chem. Ber.* **1961**, *94*, 2271.
(34) Scheneider, F. *Hoppe-Seyler's Z. Physiol. Chem.* **1964**, *338*, 131.
(35) (a) Colman, P. M.; Freeman, H. C.; Guss, J. M.; Murata, M.; Norris, V. A.; Ramshaw, J. A. M.; Venkatappa, M. P. *Nature (London)* **1978**, *272*, 319. (b) Penfield, K. W.; Gay, R. R.; Himmelwright, R. S.; Eickman, N. C.; Norris, V. A.; Freeman, H. C.; Solomon, E. I. *J. Am. Chem. Soc.* **1981**, *103*, 4382. (c) Gray, H. B.; Solomon, E. I. In *Copper Proteins*; Spiro, T. G., Ed.; Wiley: New York, 1981; p 6.
(36) (a) Adam, E. T.; Stenkamp, R. E.; Sieker, L. C.; Jensen, L. H. *J. Mol. Biol.* **1978**, *123*, 35. (b) Adman, E. T.; Jensen, L. H. *Isr. J. Chem.* **1981**, *21*, 8.
(37) (a) Richardson, J. S.; Thomas, K. A.; Rubin, B. H.; Richardson, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 1349. (b) Pantoliano, M. W.; Valentine, J. S.; Mammone, R. J.; Scholler, D. M. *J. Am. Chem. Soc.* **1982**, *104*, 1717. (c) Pantoliano, M. W.; Valentine, J. S.; Nafie, L. A. *J. Am. Chem. Soc.* **1982**, *104*, 6310.
(38) Ulrich, E. L.; Markley, J. L. *Coord. Chem. Rev.* **1978**, *27*, 109.
(39) (a) Bereman, R. D.; Kosman, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 7322. (b) Winkler, M. E.; Breman, R. D. *J. Am. Chem. Soc.* **1980**, *102*, 6244.
(40) (a) Palmer, G.; Babcock, G. T.; Vickery, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2206. (b) Malmstrom, B. G. In *Metal Ion Activation of Dioxygen*; Spiro, T. G., Ed.; Wiley: New York, 1980; p 181.
(41) (a) Fee, J. A. *Struct. Bonding (Berlin)* **1975**, *23*, 1. (b) Dawson, J. H.; Dooley, D. M.; Clark, R.; Stephens, P. J.; Gray, H. B. *J. Am. Chem. Soc.* **1979**, *101*, 5046.
(42) (a) Freedman, T. B.; Loehr, J. S.; Loehr, T. M. *J. Am. Chem. Soc.* **1976**, *98*, 2809. (b) Solomon, E. I.; Penfield, K. W.; Wilcok, D. E. *Struct. Bonding (Berlin)* **1983**, *53*, 1. (c) Solomon, E. I. In *Copper Coordination Chemistry: Biochemical and Inorganic Perspectives*; Karlin, K. D., Zubieta, J., Eds.; Adenine: New York, 1983; p 1.
(43) Appleton, D. W.; Sarkar, B. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 1686.
(44) Abderhalden, E.; Geidel, W. *Fermentforschung* **1930**, *12*, 518.
(45) Kojima, Y. *Transition Met. Chem. (Weinheim, Ger.)* **1979**, *4*, 269.

solutions under study. Titrations of HNO_3 were performed before and after each set of experiments to convert readings to pH and to calculate K values. The ionic strength was maintained at 0.1 mol dm^{-3} (KNO_3). The analytical concentration of Cu^{2+} was varied from 2.5×10^{-3} to 8.5×10^{-3} mol dm^{-3}. Four different Cyhis:Cu ratios (between 1.03:1 and 3.7:1) were employed.

Calorimetric Titrations. The calorimetric data were obtained by titration calorimetry using a Tronac isoperibol calorimeter (Model 458) equipped with a 25-mL reaction vessel. The calorimeter calibration was checked by THAM titrations with HCl and HNO_3 titrations with KOH.[46,47]

The heats of protonation of the ligands were determined by titrating appropriate solutions with standard HCl (0.2 mol dm^{-3}). The concentrations ranged from 8 to 9 mmol dm^{-3} and from 5 to 6 mmol dm^{-3} for Im and Cyhis, respectively. Heats of complex formation were measured by titrating HCl (0.2 mol dm^{-3}) into a solution containing Cu^{2+} and Im or by adding $Cu(NO_3)_2$ (0.2 mol dm^{-3}) to a solution containing Im. In the first case, the Cu^{2+} concentration varied from 2 to 3 mmol dm^{-3} and Im concentrations ranged from 7 to 10 mmol dm^{-3}. Thus, a large ligand:metal ratio was investigated.

To measure Cu^{2+}–Cyhis heats of reaction, solutions containing Cu^{2+} (ranging from 4 to 6 mmol dm^{-3}) and Cyhis (ranging from 4 to 8 mmol dm^{-3}) were titrated with HCl. Cyhis:Cu^{2+} ratios between 1 and 2 were investigated. For both protonation and complexation heats, the titrations were performed in duplicate and corrected for the dilution heats determined in separate experiments. The ionic strength was 0.1 mol dm^{-3} (KNO_3).

ESR spectra were measured with a conventional EPR X-band spectrometer (Bruker Model 200D) operating at 9.3–9.5 GHz using a 100-kHz field modulation and a 10-in. electromagnet. Quartz tubes were used for frozen solutions, while a Bruker quartz cell was employed to collect room-temperature solution spectra. The microwave frequency was calibrated with the use of powdered DPPH samples ($g = 2.0036$), while the magnetic field was carefully measured during any spectrum scan by means of a Bruker NMR gauss meter (Model ER 035 M). Values of $g_\parallel$ and $A_\parallel$ were determined directly from experimental spectra recorded on an enlarged scale, while $g_\perp$ and $A_\perp$ parameters were obtained by a simulation procedure using standard programs.

Calculations. Calculations of electrode system $E°$ values, ligand purities, protonation constants, and HNO_3 excess in the metal ion stock solutions were performed by the least-squares computer probram ACBA.[48] Calculation of the formation constants of the Cu^{2+} complexes was performed by the least-squares computer program MINIQUAD.[49] The species distribution as a function of the pH was obtained by using the computer program DISDI.[50] The heats of protonation and complexation were calculated by the least-squares computer program DOEC.[51] Errors are expressed as 3 times the standard deviation.

Results

The reaction of Cyhis with H^+ and Cu^{2+} is represented in eq 1, where L is the neutral form of the ligand with charges omitted for simplicity. The stability constant β_{mlh} for eq 1 is defined by

$$mCu + lL + hH = Cu_m(L_lH_h) \quad (1)$$

eq 2. The consecutive protonation of Im is expressed by eq 3

$$\beta_{mlh} = [Cu_m(L_lH_h)]/[Cu]^m[L]^l[H]^h \quad (2)$$

and 4 in two steps. log K, $\Delta H°$, and $\Delta S°$ values for the systems

$$H^+ + Im = HIm^+ \quad (3)$$

$$2H^+ + Im = H_2Im^{2+} \quad (4)$$

studied together with earlier values are given in Tables I and II. Previous data[15] are in good agreement with the present results,

Table III. log K Values for the Interaction of H^+ with the Imidazole Groups of Cyclopeptides of Histidine

ligand[a]	log K	ref	ligand[a]	log K	ref
c-L-His-Gly	6.30	34	*c*-L-His-L-Thr	6.57	54
	6.3	55	*c*-L-His-L-Tyr	6.48	34
c-L-His-L-Leu	6.40	34	*c*-L-His-L-Phe	6.50	34
c-L-His-L-Ser	6.27	34	*c*-L-His-L-Met	6.21	34
	6.4	54	*c*-L-His-L-Asp	6.43	34
	6.55	55	*c*-L-His-D-Thr	6.57	54
c-L-His-L-Thr	6.59	34	*c*-L-His-D-Ser	6.35	54

[a] Abbreviations: His = histidine; Gly = glycine; Leu = leucine; Ser = serine; Thr = threonine; Tyr = tyrosine; Phe = phenylalanine; Met = methionine; Asp = aspartic acid.

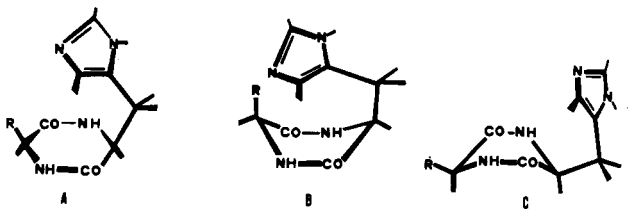


Figure 1. Planar (A), flagpole (B), and bowsprit boat (C) conformations of the diketopiperazine ring in a cyclic dipeptide.

allowing for different experimental conditions.

The equilibria needed to fit the experimental titration curves for the reaction of Cu^{2+} with Cyhis are given by eq 5–7. A simpler

$$Cu^{2+} + L = [CuL]^{2+} \quad (5)$$

$$Cu^{2+} + 2L = [CuL_2]^{2+} \quad (6)$$

$$2Cu^{2+} + 2L = [Cu_2(L_2H_{-2})]^{2+} + 2H^+ \quad (7)$$

model has been proposed for the Cu^{2+}–Cyhis system by Kojima,[15] who took into account only the $[CuL]^{2+}$ and $[CuL_2]^{2+}$ species. The different speciations are probably due to the different ligand to metal ratios and the narrower concentration and pH ranges used. The different ionic strengths probably account in part for the difference in the stability constants (Table II).

Discussion

Peptide Protonation Constants. The pK value for the first protonation step in Cyhis (6.53, Table II) is 0.5 log unit lower than that for the first protonation step in Im (7.01, Table I). In contrast, Bim, a ligand containing two Im moieties,[53] shows greater nitrogen basicity in the first protonation step then does Im (7.39 vs. 7.01). This seems to rule out any explanation of this effect based on the presence of the second nitrogen atom. Comparison of the log K data in Table III with those in Table I shows that the nitrogen basicity of Im moieties in cyclic dipeptides of histidine is always lower[34,54,55] than that of Im. Neither polar (Asp) nor nonpolar (Phe) residues cause significant changes in the pK values of the Im group of the cyclopeptides. This decreased basicity is a characteristic of the presence of the Im moiety in the diketopiperazine (DKP) backbone uninfluenced by the presence of other peptide substituents. On the other hand, Kopple et al.[56,57] demonstrated that, in cyclic dipeptides with aromatic or pseudoaromatic substituents, the favored conformer in solution has the aromatic ring folded against the DKP ring (see Figure 1). To achieve this folded conformation, the DKP ring changes from the flagpole boat conformation, normal for the monosubstituted cyclopeptide, to the planar conformation, more common for cyclic dipeptides with two aromatic residues. If both amino acids have the same configuration, the planarity of the DKP ring removes

(46) Grenthe, E.; Ots, H.; Ginstrup, O. *Acta Chem. Scand.* **1970**, *24*, 1067.
(47) Hale, J. D.; Izatt, R. M.; Christensen, J. J. *J. Phys. Chem.* **1963**, *67*, 2605.
(48) Arena, G.; Rizzarelli, E.; Sammartano, S.; Rigano, C. *Talanta* **1979**, *26*, 1.
(49) Gans, P.; Vacca, A.; Sabatini, A. *Inorg. Chim. Acta* **1976**, *18*, 237.
(50) Arena, G.; Rigano, C.; Sammartano, S. *Ann. Chim. (Rome)* **1978**, *68*, 693.
(51) Rigano, C.; Rizzarelli, E.; Sammartano, S. *Thermochim. Acta* **1979**, *33*, 211.
(52) James, B. R.; Williams, R. J. P. *J. Chem. Soc.* **1961**, 2007.
(53) Drey, C. N. C.; Fruton, J. S. *Biochemistry* **1965**, *4*, 1258.
(54) Ptak, M.; Heitz, A.; Dreux, M. *Biopolymers* **1978**, *17*, 1129.
(55) Ziauddin, Kopple, D. D.; Bush, C. A. *Tetrahedron Lett.* **1972**, *6*, 483.
(56) Kopple, K. D.; Marr, D. H. *J. Am. Chem. Soc.* **1967**, *89*, 6193.
(57) Kopple, K. D.; Ohnishi, M. *J. Am. Chem. Soc.* **1969**, *91*, 962.

side-chain interference while maintaining maximum interaction between each substituent and the DKP ring. In this case, the noncovalent interaction of each Im with the DKP ring is not as intimate as in other cyclic dipeptides in which the DKP ring is in a boat conformation and the side chains occupy axial positions (i.e., a flagpole site).

Cyhis in its neutral form resembles the cyclic anhydride of tyrosine, in that the α–β coupling constants and the upfield shifts of the β protons indicate the likelihood of a conformation in which the Im rings are sharing space over the DKP ring.[55] In addition, the ultraviolet absorption spectra indicate side-chain interactions in the cyclopeptide at pH 8.[55] Some data[57,58] suggest that protonation of the Im moiety in Cyhis results in a change of the folded conformation. This conformational change and the consequent destabilization are consistent with the decrease of pK values of the Im residues of cyclopeptides with respect to those for Im. However, pK values are a function of both $\Delta H°$ and $\Delta S°$ and therefore cannot be interpreted correctly without a knowledge of these quantities (Table II).

The protonation of the Im nitrogen (Table I) is more enthalpically and less entropically stabilized than is that of the Im nitrogen of Cyhis (Table II). These trends are consistent with the expected decreased interaction between the side-chain groups and the DKP ring upon protonation of the Cyhis. Proton NMR studies[56] show that the folded form is favored over other possible conformations of the side chains by an enthalpy change averaging −3 kcal mol^{-1}. The conformation change upon protonation results in an unfavorable enthalpy contribution. The $\Delta S°$ value associated with the equilibrium unfolded = folded (ca. −4 cal deg^{-1} mol^{-1}) corroborates this interpretation. In addition to conformational energies, the stacking interaction occurring between the unprotonated Im ring and the DKP is important to consider. We have shown that this kind of interaction is enthalpically favored and entropically unfavored.[32] Upon protonation, the enthalpic and entropic changes due to conformational change are counterbalanced by changes due to the "stacking" interaction of the unprotonated Im against the DKP ring. This effect may be enhanced by the change from "planar" to "boat" conformation of the DKP ring. The decrease in pK values of the second Im group (Table II) is 1.5 log units compared to the value for the Im nitrogen (Table I). The same is true for di-4-imidazolylmethane and other noncyclic Im derivatives. In the latter case, the effect is attributed at least in part to the presence of an adjacent positively charged amino group.[59,60] The decreased enthalpy contribution can be further attributed to the removal of the second Im ring from the space over the DKP ring.

In conclusion, our thermodynamic data, together with those previously reported for the folded–unfolded equilibrium, help elucidate the driving force that stabilzes the folded conformation, i.e., the interaction between the DKP ring and the heteroaromatic side chain. In the past, the main force has been thought to be the interaction of the two amide dipoles with the polarizable electron system of the side chain,[56] even though solvent effects were not consistent with such a hypothesis.[57] Also excluded were π–π donor–acceptor,[61] hydrophobic,[62,63] and solvophobic[64] effects. The possible involvement of solvophobic forces was rejected since the folded form turned out to be entropically unfavored while such forces were thought to be entropy driven.[56] On the contrary, we have demonstrated recently that the intramolecular solvophobic interaction accompanying the protonation of some linear dipeptides having aliphatic and aromatic side chains is enthalpically stabilized and entropically unfavored.[65,66] These experimental findings agree with Sinanoglu's theoretical conclusions.[64]

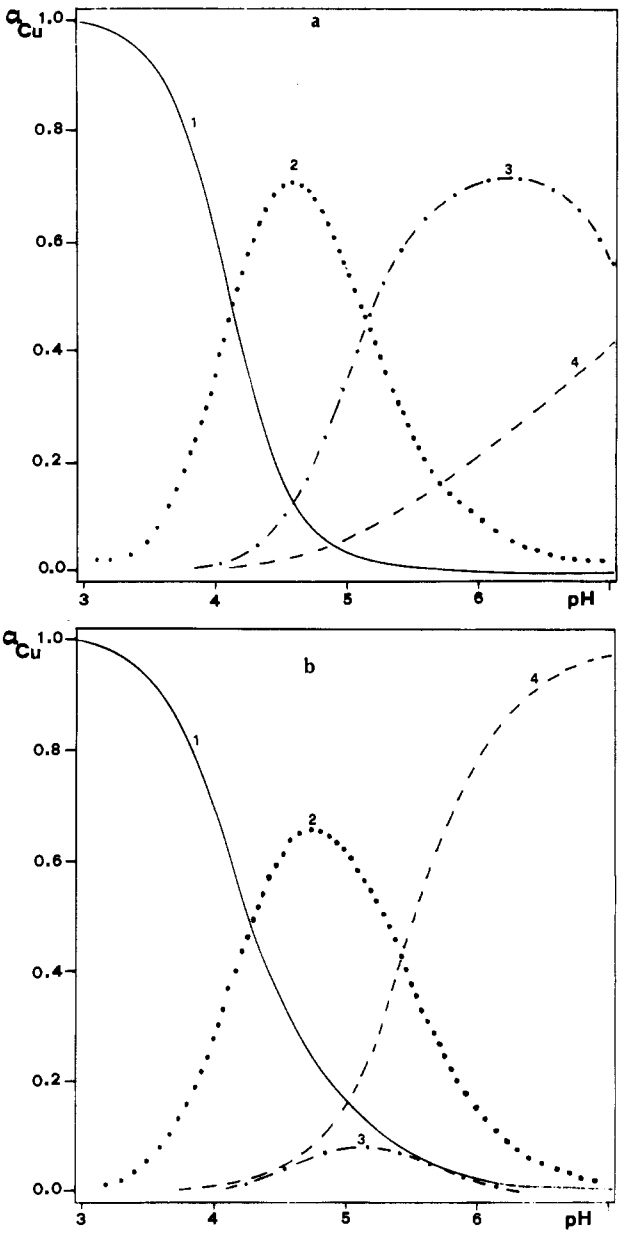


Figure 2. Species distribution diagram for the Cu–Cyhis system: (a) system with $[Cu^{2+}] = 4 \times 10^{-3}$ mol dm^{-3} and [Cyhis] = 8×10^{-3} mol dm^{-3} showing (1) free Cu^{2+}, (2) $[Cu(Cyhis)]^{2+}$, (3) $[Cu(Cyhis)_2]^{2+}$, and (4) $[Cu_2(Cyhis)_2H_{-2}]^{2+}$; (b) system with $[Cu^{2+}]$ = [Cyhis] = 6×10^{-3} mol dm^{-3} showing (1) free Cu^{2+}, (2) $[Cu(Cyhis)]^{2+}$, (3) $[Cu(Cyhis)_2]^{2+}$, and (4) $[Cu_2(Cyhis)_2H_{-2}]^{2+}$.

Cu^{2+}–Cyclopeptide Association Constants. Distribution diagrams are shown in parts a and b of Figure 2 for copper:Cyhis ratios of 1:2 and 1:1, respectively. The equilibrium Cu^{2+} + Cyhis = $[Cu(Cyhis)]^{2+}$ is enthalpically favored and entropically unfavored (Table II). The $\Delta H°$ value for $[Cu(Cyhis)]^{2+}$ formation is significantly less negative than that for $[Cu(Im)_2]^{2+}$ formation (Table I), where two nitrogens are involved in the coordination, but more negative than that for $[Cu(Im)]^{2+}$ formation. This result raises questions as to whether Cyhis coordinates via one or two nitrogen atoms. It is possible that two nitrogens are coordinated to Cu^{2+} and that the difference in the $\Delta H°$ values for $[Cu(Cyhis)]^{2+}$ formation (−9.88 kcal mol^{-1}) and $[Cu(Im)_2]^{2+}$ formation (−13.4 kcal mol^{-1}) is due to the formation of an unusually large chelate ring in the former case. The $\Delta H°$ value for the equilibrium $[Cu(Cyhis)]^{2+}$ + Cyhis = $[Cu(Cyhis)]_2^{2+}$, −7.1 kcal mol^{-1}, sug-

(58) Grebow, P. E.; Hooker, T. M., Jr. *Biopolymers* **1974**, *13*, 2349.
(59) Tanokura, M.; Tasumi, M.; Miyazawa, T. *Biopolymers* **1976**, *15*, 393.
(60) Boschov, P.; Seidel, W.; Muradian, J.; Tominaga, M.; Paiva, A. C. J.; Juliano, L. *Bioinorg. Chem.* **1983**, *12*, 34.
(61) Ziauddin; Kopple, K. D. *J. Org. Chem.* **1970**, *35*, 253.
(62) Kauznann, W. *Adv. Protein Chem.* **1959**, *14*, 34.
(63) Ben-Naim, A. *Hydrophobic Interaction*; Plenum: New York, 1980.
(64) Sinanoglu, O. *Int. J. Quantum Chem.* **1980**, *18*, 381.
(65) Impellizzeri, G.; Bonomo, R. P.; Cali, R.; Cucinotta, V.; Rizzarelli, E. *Thermochim. Acta* **1984**, *72*, 263.
(66) Impellizzeri, G.; Bonomo, R. P.; Cali, R.; Cucinotta, V.; Rizzarelli, E. *Thermochim. Acta* **1984**, *80*, 275.

Table IV. EPR Parameters Associated with $[Cu(Cyhis)]^{2+}$ and $[Cu(Cyhis)_2]^{2+}$ Complexes in Aqueous Solution

	$[Cu(Cyhis)]^{2+}$	$[Cu(Cyhis)_2]^{2+}$
$g_\parallel$		2.253 (2)
$A_\parallel$, cm^{-1}		0.0190 (2)
$g_\perp$		2.050 (5)
$A_\perp$, cm^{-1}		0.0030 (4)
$A_\parallel^N$, cm^{-1}		0.0016 (2)
$A_\perp^N$, cm^{-1}		0.0012 (2)
g_{iso}	2.146 (5)	2.118 (2)
A_{iso}, cm^{-1}	0.0068 (2)	0.0082 (2)
A_{iso}^N, cm^{-1}	0.0013 (2)	0.0014 (2)

gests that at least one more nitrogen atom coordinates in the latter case.

The formation of $[Cu_2(Cyhis)_2H_{-2}]^{2+}$ is both enthalpically and entropically favored. No similar species for the Im system has been found. Recently, the structure of a Cu^{2+}–Cyhis dimeric species has been reported.[20] According to this structure, one Cu^{2+} is coordinated to four nitrogen donor atoms, i.e., two Im nitrogens and two deprotonated peptide nitrogens of different molecules. The remaining Cu^{2+} binds the other two Im nitrogens and two carbonyl oxygens in the DKP rings of two different ligand molecules; a water molecule occupies the fifth coordination site. Considering that Brubaker et al.[13] suggest that the cyclopeptide nitrogen deprotonation assisted by Cu^{2+} complexation may occur at relatively low pH values, we hypothesize that the species maintains the same structure in solution. The exothermic contribution due to the coordination of four Im nitrogens is counterbalanced by the endothermic contribution of deprotonation of the peptide nitrogens of the DKP rings. Usually, in Cu^{2+} complexes of linear dipeptides the formation of $CuLH_{-1}$ species, in which one amine nitrogen, one peptide nitrogen, one carboxylic oxygen, and a water molecule coordinate to Cu^{2+}, is endothermic.[67] Keeping in mind the structure found for solid dimeric species of Cu^{2+},[20] the planar conformation of the cyclopeptide ring may provide an interaction between the Im ring and the DKP rings, with a consequent favorable enthalpic contribution.

EPR. EPR measurements were carried out to specifically answer the question of how many nitrogen atoms are coordinated to Cu^{2+}. When one deals with a system where more than one species can be present at the same time, experimental conditions must be chosen in such a way as to maximize the concentration of a single species. The species distribution diagram is useful in this regard since it allows one to determine the conditions under which a spectrum must be run.

The potentiometric data show that three species are formed by the interaction of Cu^{2+} with Cyhis, i.e., $[Cu(Cyhis)]^{2+}$, $[Cu(Cyhis)_2]^{2+}$, and $[Cu_2(Cyhis)_2H_{-2}]^{2+}$. Well-resolved spectra are obtained easily when the ligand to metal ratio is greater than 2; $[Cu(Cyhis)_2]^{2+}$ is in fact the only species formed around pH 6. Parts a and b of Figure 3 show the EPR room- and low-temperature spectra of the solutions containing the $[Cu(Cyhis)_2]^{2+}$ species. The spectral resolution could not be enhanced by the addition of methanol or ethanol since the addition of these solvents causes ligand precipitation. Nevertheless, in both spectra the superhyperfine pattern arising from the interaction of the copper(II) odd electron with nitrogen atoms is quite evident. The room-temperature spectrum is peculiar because a large line-width variation only allows the highest field line to be resolved. It is well-known that with high-molecular-weight complexes such a pattern is often observed. Actually, the line shape of the EPR room-temperature spectra is strongly dependent on the tumbling of molecules. Other examples of such behavior are $Cu(dopa)_2$[68] and Cu(his)(ala).[69] However, even under these conditions a nine-line pattern can be observed and is attributed to the coordination of four nitrogen atoms to copper(II) (see Figure 3a). Spin Hamiltonian parameters derived from the EPR spectra are reported in Table IV. The ones concerning the bis complex are identical with those obtained from the spectrum of $[Cu(Cyhis)_2(ClO_4)_2]$ powder,[70] which contains discrete $[Cu(Cyhis)_2]^{2+}$ units. These data deserve a few words; in fact the $g_\parallel$ and $a_\parallel$ values are similar to those obtained for Cu^{2+}–dipeptide systems,[71] where a CuN_2O_2 chromophore is formed, rather than to those expected for four-nitrogen coordination. These values are strongly indicative of a tetrahedral distortion of the basal plane as has also been observed by means of diffractometry on $[Cu(Cyhis)_2(ClO_4)_2]$ crystals.[22]

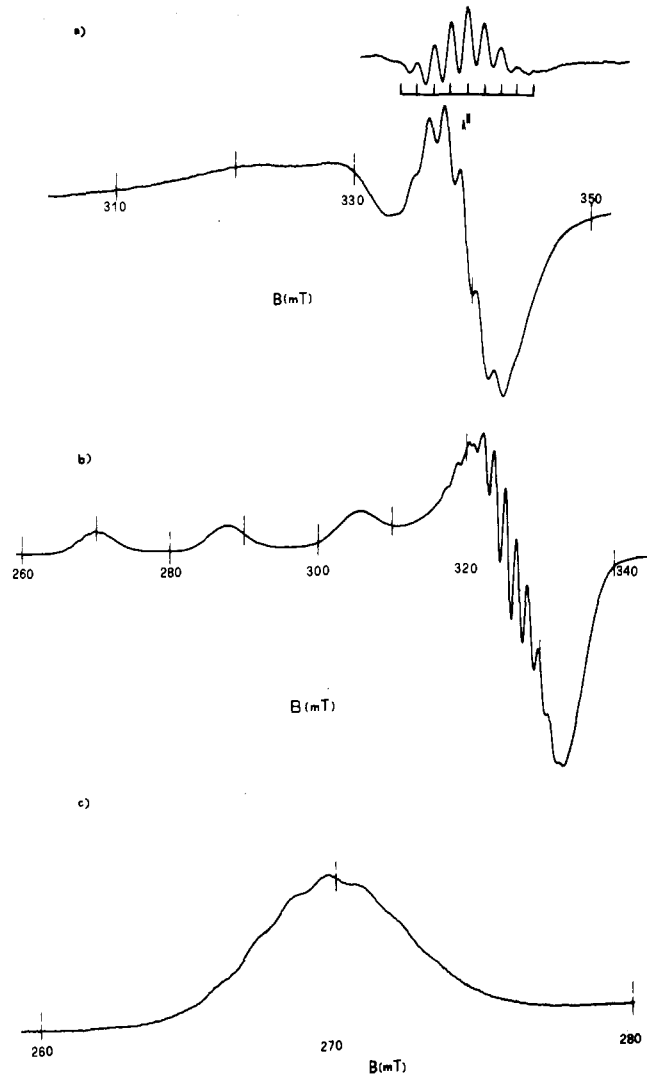


Figure 3. Aqueous solution EPR spectra of 5×10^{-3} mol dm^{-3} $[Cu(Cyhis)_2]^{2+}$ (microwave frequency 9.725 GHz, microwave power 10 mW, field modulation intensity 5 G, time constant 0.3 s): (a) room temperature solution (insert shows second-derivative mode the $-^3/_2$ line); (b) frozen solution at 150 K; (c) nitrogen superhyperfine pattern of the low-field line at 150 K.

Unfortunately, it is difficult to obtain quantitative information on the mono complex, $[Cu(Cyhis)]^{2+}$. In fact, when the metal:ligand ratio is 1:1 (see Figure 2b), $[Cu(Cyhis)]^{2+}$ reaches its maximum of formation (65%) around pH 4.8. Obviously, the room-temperature EPR spectrum reflects this situation, and its interpretation is therefore complicated by the presence of other absorbing species. The resolution of the spectrum is not an ideal one; Figure 4a shows the best result we were able to obtain. The second-derivative mode on the $-^3/_2$ line shows a five-peak pattern due to two nitrogens and thus supports the coordination to Cu^{2+}

(67) Gergely, A.; Nagypal, I. *J. Chem. Soc., Dalton Trans.* **1977**, 1104.
(68) Hyde, J. S.; Froncisz, W. *Annu. Rev. Biophys. Bioeng.* **1982**, *11*, 391.
(69) Huet, J.; Vilkas, E. *Inorg. Chim. Acta* **1984**, *91*, 43.
(70) Schugar, H. J.; Fawcett, T. G.; Hendrickson, D. N.; Felthouse, T. R. *Inorg. Chem.* **1978**, *17*, 2707.
(71) Bonomo, R. P.; Cali, R.; Cucinotta, V.; Impellizzeri, G.; Rizzarelli, E. *Inorg. Chem.* **1986**, *25*, 1641.

Table V. Thermodynamic Functions[a] for the Possible Chelate Effect of *cyclo*-(L-Histidyl-L-histidyl) with Copper(II) Ions

ΔN	reacn	$\Delta G°$, kcal mol^{-1}	$\Delta H°$, kcal mol^{-1}	$\Delta S°$, cal mol^{-1} deg^{-1}	$\Delta G°'$,[b] kcal mol^{-1}	$\Delta S°'$,[c] cal mol^{-1} deg^{-1}
1	$[Cu(Im)_2]^{2+}$ + Cyhis = $[Cu(Cyhis)]^{2+}$ + 2Im	2.2	3.5	−4	4.6	−12
2	$[Cu(Im)_4]^{2+}$ + 2Cyhis = $[Cu(Cyhis)_2]^{2+}$ + 4Im	2.3	6.6	−14	7.0	−30

[a] Nancollas, G. H. *Interactions in Electrolyte Solutions*; Elsevier: New York, 1966; pp 117–164. [b] $\Delta G°' = \Delta G° + RT \ln 55.5 = \Delta G° + 2.36(\Delta n)$. [c] $\Delta S°' = \Delta S° - (\Delta N)R \ln 55.5 = \Delta S° - 7.9(\Delta n)$.

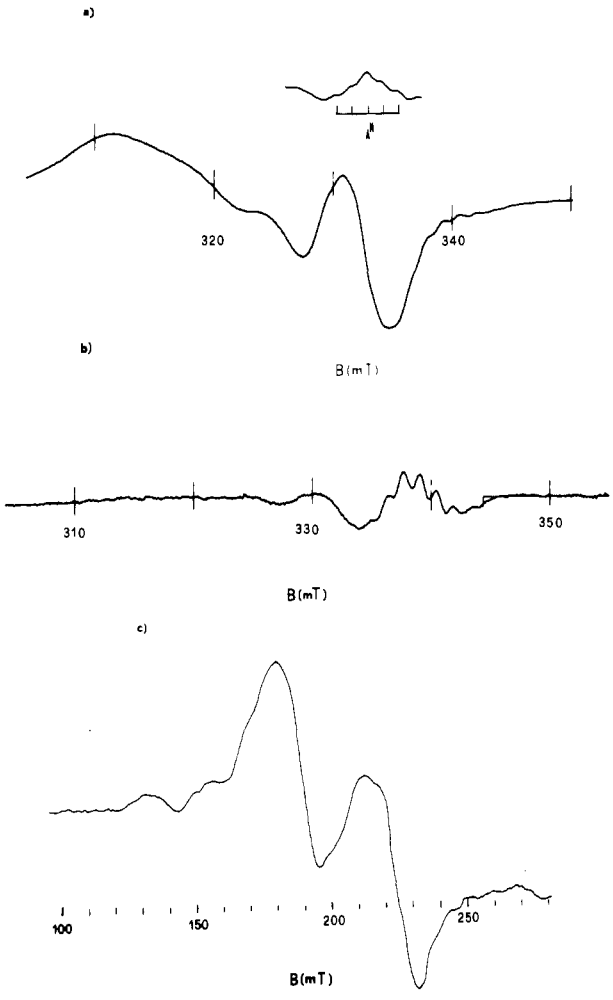


Figure 4. Aqueous solution EPR spectra of the copper(II) ion and Cyhis in a 1:1 ratio (2×10^{-3} mol dm^{-3}): (a) room-temperature solution, mode of the $-^3/_2$ line (microwave frequency 9.725 GHz, microwave power 26 mW, field modulation intensity 10 G, time constant 0.3 s); (b) room-temperature solution, pH 6.8 (microwave frequency 9.725 GHz, microwave power 210 mW, field modulation intensity 12 G, time constant 0.5 s); (c) frozen solution, pH 6.8 at 77 K (instrument conditions as for (b)).

of both nitrogens of the Im residues in the $[Cu(Cyhis)]^{2+}$ species.

Figure 4b,c shows the spectra run on a solution with a copper:ligand ratio of 1:1 but at a higher pH, namely 6.8. At this pH value, the signal due to the mono complex disappears and the spectrum reveals the features of the bis complex, the lines of which can be seen at both room and low temperature. (Owing to the very low concentration of this species, high receiver gain and klystrom power levels had to be employed to observe its signal under the above experimental conditions.) This was surprising since we knew through potentiometry that at this pH the majority of Cu^{2+} forms a dimeric species (see distribution diagram). There is very likely a problem due to the ratio between signal intensity and dimer concentration[72] that makes the detection of this dimer species at 3 kG difficult. We could not increase the concentration of both ligand and metal ion owing to precipitation problems. Nevertheless, the presence of an EPR signal due to a dimeric species is revealed at half-field when one looks for the ($\Delta M_s = \pm 2$) transition at low temperature.

The EPR data make it possible to understand better the thermodynamic data. As noted above, the relatively low enthalpic contribution to the formation of Cyhis complexes can be ascribed to the difficulty in forming chelate rings with such a high number of atoms. Three possible structures are 11-, 12-, and 13-membered chelate rings, through N(1) and N(5), N(1) and N(6), and N(2) and N(6), respectively. According to the data shown in Table V, the chelate effect[73] can be ruled out. It is possible, however, that the thermodynamic parameters are influenced by the conformational change upon complexation. In the solid state, Cyhis has been found to have a bowsprit conformation when complexing with Cu^{2+} (see Figure 1).[22] Though solid-state and solution data should be correlated cautiously, the ligand may also have a bowsprit conformation in solution to better coordinate the Cu^{2+}. This conformation minimizes the interaction of the Im residues with the DKP ring, yielding an unfavorable enthalpic contribution. The contribution should not be lower than 3 kcal mol^{-1}, which is the average value reported in the literature[56] for the folded–unfolded equilibrium for cyclopeptides with an aromatic substituent such as *cyclo*-(glycylhistidine) or *cyclo*-(glycylphenylalanine).

Conclusions

Cyhis coordination demonstrates unusual behavior: (i) The formation of unusual-size chelate rings is driven by the peculiar conformational characteristics of the DKP ring. (ii) In the dimer species, the peptide nitrogen deprotonation due to Cu^{2+} complexation is enhanced by the Im nitrogen coordination and by the interaction between the DKP and the Im rings. Taken together, these factors account for the lowering of the deprotonation pH. In fact, this pH is much lower than that found for other cyclopeptides.[11] (iii) The thermodynamic parameters of complexation of Cyhis with H^+ and with Cu^{2+} seem to depend mainly upon the extent of intramolecular interaction of the DKP ring with the Im residues and of the side chains with each other. A knowledge of the effect of the geometric requirements of different metal ions (e.g., Cu^{2+} and Zn^{2+}) on these noncovalent interactions[74] might help in clarifying some data concerning selective coordination encountered in biological systems.

Acknowledgment. We thank the CNR (Rome) and the Ministry of Public Education (Rome) for partial support of this work.

(72) Dunhill, R. A.; Pilbrow, J. R.; Smith, T. D. *J. Chem. Phys.* **1986**, *45*, 1474.
(73) Schwarzenbach, G. *Helv. Chim. Acta* **1952**, *35*, 2344.
(74) Frieden, E. *J. Chem. Educ.* **1975**, *52*, 754.