Binding of Copper(II) to Potentially Tridentate Amino Acid Ligands[1]

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Abstract: An extensive study of ultraviolet charge-transfer spectra, visible circular dichroism (CD) and absorption spectra, titrations, and formation constants is exploited to ascertain the mode of copper(II) binding to a variety of potentially tridentate ligands. In the series of α,ω-L-diaminocarboxylate anions, lysine and ornithine bind to copper(II) as bidentate substituted glycines. 2,4-Diaminobutyrate complexes in this mode and also as a tridentate ligand with either one nitrogen and one oxygen or two nitrogen donors in the chelate plane. 2,4-Diaminopropionate binds as a tridentate ligand with an apical carboxylate group. The corresponding zero net charged α,ω-diaminocarboxylic acids all bind as substituted glycines with unbound ω-ammonium groups. Similarly, the neutral histidine molecule binds to copper(II) through the glycine locus with an unbound protonated imidazolium group. Though dependent as in some of the above cases on the other ligands bound, the first histidinate anion serves mainly as a tridentate ligand with primary bidentate nitrogen donors in the chelate plane and a weaker apical coordination of the carboxylate group. The second histidinate anion binds as a bidentate ligand. The CD of copper(II) chelates of histidine containing dipeptides is nearly an additive function of independent contributions from amino and carboxyl terminal amino acid residues. Carnosine exhibits the largest CD magnitudes among the dipeptide complexes. Titration and CD evidence are presented for the occurrence in solution of the unique dimer structure found for carnosine in the solid.

Potentially tridentate ligands such as the amino acid histidine may serve as bidentate chelates by three different combinations of pairs of donor atoms and as tridentate chelates. Thus uncertainties arise in determining bonding modes. The uncertainties are compounded when the metal ion is copper(II). Coordination compounds of copper(II) typically consist of four nearby donor atoms arranged approximately in a plane about the metal ion, with the possibility of one or two more distant axial donors. All three donor atoms of tridentate ligands such as histidine are sterically prohibited from simultaneously occupying planar positions about a single metal ion. Since each of the three donor groups may be placed in a unique apical position, three different tridentate binding modes also exist for copper(II). Most of these six possible combinations have been suggested many times for the binding of histidine to copper(II). Important variables such as pH and ligand-to-metal ion ratios have often not been considered. The approach of this paper is to study, under controlled solution conditions, a variety of tridentate and bidentate ligands with copper(II) so that some complexes may serve as models for others. Molecules studied include α-amino acids, α,ω-diaminocarboxylic acids, histidine, and some histidine derivatives, including dipeptides.

We wish to bring as many experimental techniques as possible to bear on the problems of tridentate ligand binding to copper(II) in solution. Infrared studies were considered and dropped because, apart from the difficulties associated with aqueous solutions, interpretations not only differ from those of other methods, but authors employing infrared techniques often disagree. In addition authors of infrared papers on the amide bond have consistently and wrongly labeled the nitrogen more basic than the oxygen, leading to incorrect structures for proton and metal ion binding sites.[2] It is well established by nuclear magnetic resonance[3] and X-ray diffraction[4] studies that the oxygen is the site of both protonation and metal ion coordination to the neutral amide function, while the nitrogen becomes the metal ion binding site if ionization of an amide hydrogen occurs.

Experimental techniques incompletely exploited include calorimetric measurements and Raman spectra, which are better suited to aqueous solutions than infrared spectra. Electron spin resonance investigations of copper(II) complexes have been useful, but the interpretation is hindered by uncertain symmetries and theory that is difficult to apply.

We undertook an extensive investigation of selective broadening of ligand peaks in proton magnetic resonance spectra by traces of paramagnetic copper(II)[5] in an attempt to elucidate sites of binding. Linear plots of peak width *vs.* copper(II) concentration were obtained. Our conclusion is that the method is equivocal for small ligands such as appear in Table II and no results are reported. Selective broadening by paramagnetic ions should, however, prove useful for determination of site binding, at high ligand-to-metal ion ratios, for complex ligands such as small peptides and proteins. The binding modes deduced may not always apply to lower ligand-to-metal ion ratios.

The experimental observables that proved most useful in this study of potentially tridentate ligand binding to copper(II) in solution are ultraviolet ligand to metal ion charge transfer spectra, visible spectra and circular dichroism through the ligand-field bands of copper(II),

(1) This research was supported by a grant from the National Science Foundation.

(2) For a critique see A. R. Katritzky and R. A. Y. Jones, *Chem. Ind. (London)*, 722 (1961).

(3) G. Fraenkel, A. Lowenstein, and S. Meiboom, *J. Phys. Chem.*, **65**, 700 (1961); D. Herbison-Evans and R. E. Richards, *Trans. Faraday Soc.*, **58**, 845 (1962); M. J. Janssen, *Spectrochim. Acta*, 17, 475 (1961); R. Stewart and L. J. Muenster, *Can. J. Chem.*, **39**, 401 (1961).

(4) H. C. Freeman, *Advan. Protein Chem.*, 22, 257 (1967); J. D. Bell, H. C. Freeman, A. M. Wood, R. Driver, and W. R. Walker, *Chem. Commun.*, 1441 (1969).

(5) N. C. Li, R. L. Scruggs, and E. D. Becker, *J. Amer. Chem. Soc.*, **84**, 4650 (1962).

and formation constants for comparisons among bidentate binding modes.

Experimental Section

All ligands were the highest quality products available from a variety of commercial sources, and the equivalent weights were checked by titration. Titration curves were obtained on a Radiometer TTT1a pH meter with associated recorder. Spectra were recorded on Cary Model 11 or 14R spectrophotometers. Circular dichroism was measured on a Durrum-Jasco ORD-UV-5 recording spectropolarimeter with a CD attachment. All CD results are presented as differential molar absorptivities between left and right circularly polarized light per mole of copper(II), $\Delta\epsilon$. Titrations for formation constants were determined at 25.0° and 0.15 *M* ionic strength and the data analyzed by the projection strip method.[6] Solutions containing equimolar amounts of Cu(II) and each of two ligands are expected to yield 50% of mixed complex. In most cases that have been studied the proportion of mixed complex is greater than the above statistical amount.

Results

Charge-Transfer Spectra. Since the molar absorptivities at maxima for charge-transfer spectra recorded in Table I were necessarily obtained in dilute solutions

Table I. Copper(II) Charge-Transfer Spectra

	Ligand charge	nm	$10^{-3}\epsilon$
Acetate	1−	239	3.2
Ethylamine	0	235	5.7
Glycine	1−	231	6.1
Alanine	1−	234	6.7
Norvaline	1−	236	6.7
$NH_2(CH_2)_2NH_2$	0	229	6.4
$NH_2(CH_2)_3NH_2$	0	252	6.3
2,3-Diamino-	0	230	(4.1)
propionic acid	1−	231	5.8
2,4-Diamino-	0	235	(4.2)
butyric acid	1−	248	5.4
Ornithine	0	235	6.5
	1−	238	5.8
Lysine	0	235	6.7
	1−	235	6.5
Imidazole	0	~270	0.8
		235	2.4
Histamine	0	245	3.5
Histidine	1−	245	3.8

($[Cu^{2+}] \simeq 1.5 \times 10^{-4}$ *M*) where complexes may not be fully formed, excesses of bidentate ligands (*e.g.*, 3:1) were employed. Molar absorptivities in parentheses in Table I vary as the concentration of the still partially protonated ligand is increased. A continuous concentration dependence was observed with the net zero charged form of histidine. The first two ligands in Table I, both unidentate, were at 0.5 *M*. A 20:1 imidazole to copper(II) ratio was read in the sample compartment *vs.* 16 equiv of imidazole in the reference compartment, so that the ϵ refers to a 4:1 complex corrected for absorption by unbound ligand. All molar absorptivities, ϵ, in Table I are per mole of copper(II).

On the basis of a simple weighted addition of the results for acetate ion and ethylamine shown in Table I, the charge transfer (CT) spectra of 2:1 chelates of amino acids such as glycine with copper(II) are predicted to exhibit a maximum at 237 nm with ϵ 4500 in contrast to the observed maxima at 231–236 nm with ϵ 6100–6700. Thus not only are the CT spectra of copper(II) with acetate ion and ethylamine discouragingly similar in wavelength for diagnostic purposes, but also the results for monodentate complexes do not carry over to chelates where the bond angles are different. Therefore it is necessary to employ chelate models in making comparisons involving chelating ligands.

The CT spectrum of copper(II) with 1,3-diaminopropane does occur at a notably longer wavelength of 252 nm, which is also found in the 1− charged form of 2,4-diaminobutyric acid, indicating at least two nitrogen donor atoms for this negatively charged ligand. The net zero-charged ligand exhibits a CT spectrum at a wavelength similar to alanine, suggesting N and O donors in this species. Lysine appears to bind through N and O donors regardless of the ligand charge. The wavelength and magnitude for the copper(II) complex of ornithine with net zero charge indicates that the latter also chelates through N and O donors in the chelate plane. CT spectra do not seem to yield sharp distinctions for the other amino acids of this group.

The only unbound ligands in Table I yielding an absorption maximum at >200 nm contain imidazole rings, and all three display maxima from 205 to 208 nm with $\epsilon \sim 5000$. This ligand band is relatively unaffected by complexation with copper(II) being shifted slightly to longer wavelength with a modest increase in intensity. Upon complexation with copper(II), imidazole also gives weak bands as shoulders at about 270 and 235 nm with ϵ 200 and 600, respectively, for each imidazole bound. These weak bands are obscured in histamine– and histidine–copper(II) chelates by the stronger CT bands appearing as distinct shoulders near 245 nm with $\epsilon \sim 3700$. The near identity of wavelength and magnitude of this CT band in histamine and histidine suggests similar bonding to copper(II) through two nitrogen donors in both ligands. Resolution of the ligand and CT bands in the histidine–copper(II) chelate would yield for the CT absorption a wavelength too long and an intensity too small to be consistent with exclusive N and O donor systems like glycine, alanine, and norvaline.

Visible Absorption Spectra and Circular Dichroism (CD). Visible CD and absorption spectra of 1:2 complexes of copper(II) and L-amino acids each bound through one amino N and one O donor atoms exhibit negative CD and absorption near 620 nm, as typified by L-alanine.[7] From the results on L-amino acids presented in Table II this pattern is also exhibited by both charged forms of lysine, indicating that it is bound to copper(II) *via* the α-N and O atoms with the charged or uncharged ϵ-amino group projecting away from the metal ion. The zero-charged form of ornithine displays a spectrum similar to that of lysine, suggesting that the predominant binding mode is chelation through α-N and O atoms with an unbound charged δ-amino group.

Maxima in the visible absorption spectra for 1:2 copper(II) complexes of ligands each with two amino N donors occur at 540, 550, and 558 nm for 1,2-diaminopropane, 1,2-diaminoethane (en), and 1,3-diaminopropane, respectively. The CD of the 1:2 copper(II)

(6) F. J. C. Rossotti and H. S. Rossotti, "The Determination of Stability constants," McGraw-Hill, New York, N. Y., 1961, pp 99–101.

(7) J. M. Tsangaris and R. B. Martin, *J. Amer. Chem. Soc.*, 92, 4255 (1970).

Table II. Visible Circular Dichroism of Copper(II) Chelates of L-Amino Acids

	Net L-ligand charge	2:1 chelate CD nm	2:1 chelate CD Δε	2:1 chelate Absorption nm	2:1 chelate Absorption ε	1:1:1 with glycine CD nm	1:1:1 with glycine CD Δε	1:1:1 with glycine Absorption nm	1:1:1 with glycine Absorption ε	1:1:1 with en CD nm	1:1:1 with en CD Δε	1:1:1 with en Absorption nm	1:1:1 with en Absorption ε
Alanine	1−	625	−0.09	620	57	629	−0.05	620	50	615	−0.04	582	57
2,3-Diamino-propionic acid	0	658	+0.14	626	40								
	1−	548	+0.24	556	61	580	+0.09	587	50	544	+0.11	550	62
2,4-Diamino-butyric acid	0	606	+0.23	620	50								
	1−	602	+0.95	606	92	604	+0.67	605	61	570	+0.57	570	70
Ornithine	0	560	−0.07	619	56			625	51	594	−0.03	576	57
	1−	633	+0.48	631	67	633	+0.31	630	65	616	+0.15	588	57
Lysine	0	605	−0.15	618	57	615	−0.08	622	55				
	1−	600	−0.16	620	60	612	−0.08	622	57	600	−0.07	575	56
Asparagine	1−	594	−0.04	632	45	590	−0.02	624	48				
	2−	655	+0.60	580	70	660	+0.25	615	70				
		517	−0.12			520	−0.06						
										1:1:1 with histamine			
1-Methylhistidine	0	642	−0.28	659	46								
	1−	607	−0.46	626	57	618	−0.31	631	53	585	−0.46	602	65
Histidine	0	644	+0.14	645	36								
	1−	690	+0.52	640	86	644	+0.32	610	53	700	+0.24	626	82
		570	−0.03							596	−0.16		
Histidinamide	0	680	+0.75	638	84					680	+0.33	625	75
		550	+0.10							526	+0.03		
Histidinol	0	650	+0.25	600	87	674	+0.05	605	53	640	+0.10	610	72
		532	+0.20			570	−0.05			540	+0.09		

complex of (−)-1,2-diaminopropane yields $\Delta\epsilon = +0.27$ at 510 nm.[8] This ligand may be correlated with D-amino acids. Ligands derived from L-amino acids in the upper half of Table II, when bound through two N donors, possess a side chain in the same disposition as for D-amino acids. Therefore small positive CD and absorption at wavelengths shorter than 560 nm in Table II is indicative of four N donor atoms. This description applies to the negatively charged 2:1 diaminopropionate complex indicating four N donors in the plane about copper(II). The 1:1:1 diaminopropionate, en, and copper(II) complex also possesses four N donors according to the absorption maximum.

Absorption maxima at wavelengths longer than 560 nm and shorter than 610 nm indicate a mixture of oxygen and amino nitrogen donors about copper(II). The 1:1:1 complex of lysine, en, and Cu(II) exhibits an absorption maximum at 575 nm, consistent with three N and one O donor atoms. For all the L-lysine complexes in Table II, $\Delta\epsilon$/(lysine) is −0.07 to −0.08. Similarly for the L-alanine complexes $\Delta\epsilon$/(alanine) = −0.04 to −0.05. The 1:1:1 complex of diaminopropionate and glycinate shows an absorption maximum at 587 nm also consistent with three N and one O donor atoms in the chelate plane.

The 2:1 L-histidinate chelate of copper(II) exhibits a two-signed CD curve with the crossover wavelength, from a negative CD peak at shorter wavelength to a positive one at longer wavelength, at about 40 nm less than the wavelength of the absorption maximum. This uncommon behavior is also observed in the 2:1 complex of 2,4-diaminobutyric acid at a lower pH than those of Table II, in the zero-net-charge ornithine complex with glycine, and as shown in Table II in two instances with asparagine. The histidinate–histamine and histidinol–glycinate mixed complexes also exhibit a two-signed CD curve but with crossover wavelengths about 25 nm longer than the absorption maximum. Typical amino acids such as alanine cross over at about 80 nm longer than the absorption maximum.

There is considerable similarity between the CD of the 1:2 copper(II) complexes of histidinate anion and of asparagine dianion. Though weaker, asparagine monoanion exhibits CD and absorption spectra similar to those of the alanine complexes so that the neutral β-amide group is evidently only weakly coordinated if at all. Upon addition of excess base ionization of a β-amide hydrogen occurs near pH 11 (and some precipitation takes place). Since the amide ionization displays considerable promotion by copper(II) the nitrogen of the negative β-amide group must be bound to copper(II). It is possible to build a pseudochair structure for the six-membered chelate ring involving amino and planar amide nitrogens which also permits apical chelation of the α-carboxylate group. Asparagine dianion thus serves as a tridentate ligand consistent with the dramatic change in CD upon amide hydrogen ionization. The γ carbons of histidine and asparagine are both trigonally hybridized and linked to nitrogens which are coordinated to copper(II) in solutions sufficiently basic. Allowing for some reduced flexibility due to the imidazole ring, we suggest similar structures for the histidinate anion and asparagine dianion copper(II) complexes when both exhibit similar two-signed CD curves. Continuing the parallelism, we note that absorption maxima for both complexes occur at longer wavelengths than expected for four nitrogen donors about the copper(II) chelate plane. Two-to-one complexes of other amino acid amides absorb near 530 nm,[7] 50 nm shorter than asparagine dianion. The 2:1 histamine complex absorbs at 598 nm, 40 nm shorter than histidine. The longer wavelength absorptions for the potentially tridendate ligands may be ascribed to apical chelation. Substitution of apically coordinated water in copper(II) complexes by nitrogen donors is known to displace the absorption maximum to longer wavelengths.[9] The effect of apically coordinated carboxylate groups in regular or distorted geometries

(8) R. D. Gillard, *J. Inorg. Nucl. Chem.*, **26**, 1455 (1964).

does not seem to have been established. Apical chelation of a single carboxylate group in the 2,3-diaminopropionate complexes appears to increase the wavelength of the absorption maximum by about 7 nm compared to 1,2-diaminopropane. Complexes in Table II in which apical chelation is thought to occur exhibit a relatively large longer wavelength positive CD.

Formation Constants. The nature of metal ion bonding in ligands with several binding modes may be clarified by estimating the strength of binding in each mode from known formation constants of suitable model compounds. Because the basic strengths of binding sites may be different in the model compounds and the ligand of interest, the formation constants taken from model compounds require some adjustment for any changes in basicity. We shall assume that for the cases considered here a change in base strength as measured by the pK_a causes a corresponding change in the logarithm of the formation constant; *i.e.*, one unit reduction in pK_a gives a formation constant one log unit smaller. Because in our cases the adjustments are never large, no qualitative conclusions would be changed if some proportionality factor other than unity (such as 0.6) were chosen. Ratios of the formation constants will be utilized later so that errors introduced by approximations tend to cancel.

The calculated constants for formation of $CuHL^{2+}$ from neutral diaminopropionic and diaminobutyric acids,[10] ornithine, and lysine[11] are about equal to the value predicted when the ligands are considered as substituted glycines after allowance for the greater basicity of the α-amino group in glycine. Therefore the $CuHL^{2+}$ complexes for all four α,ω-diaminocarboxylic acids consist predominantly of the glycine locus in the chelate plane with the unbound positively charged ω-ammonium side chains projecting away from the metal ion.

Upon further addition of base it is evident from published titration results[12] that the ω-ammonium groups of lysine and ornithine copper(II) complexes titrate separately. Ionizations from the ω-ammonium groups in both complexes occur in pH regions that may be accounted for electrostatically; no strong binding of ω-amino groups need be postulated to satisfy the titration results. Thus both the lysine and ornithine complexes may be considered as substituted glycines for both neutral and anionic forms of the ligands, with at most only a weak apical interaction of the δ-amino group in ornithine.

In addition to a pronounced lowering of the pH region for ω-ammonium ionizations in the presence of copper(II), the observed stability constants for formation of CuL^+ for diaminopropionate and diaminobutyrate[10] are more than 20 times greater than is predictable on the basis of 1,2-diaminoethane and 1,3-diaminopropane after adjustment for the greater basicity of the models. Therefore the carboxylate groups are bound to copper(II) and contribute to the stability of the CuL^+ complexes.

For histidine there are three possible bidentate binding modes involving any two combinations of the amino, imidazole, and carboxylate groups. The carboxylate group with pK_a near 1.9 is always ionized under the conditions usually studied. For the imidazolium and ammonium nitrogen deprotonations we define the acid ionization constants

$$IH^+ \rightleftharpoons I + H^+ \qquad K_I = (I)(H^+)/(IH^+)$$

$$HG \rightleftharpoons G^- + H^+ \qquad K_G = (G^-)(H^+)/(HG)$$

The symbol G is used in the last case to designate a substituted glycine type ionization. For histidine, $pK_I = 6.1$ and $pK_G = 9.2$ at 25° and near 0.2 ionic strength.[11]

Metal ions, not present in excess, may interact with histidine to form bidentate chelates at the imidazole and carboxylate groups, amino and carboxylate groups, and imidazole and amino groups. For each of these pairs successively we write the following formation constants, where the charges apply to the immediate site of binding (the carboxylate interaction is weak in the first case).

$$M^{2+} + I \rightleftharpoons MI^{2+} \qquad K_{MI} = (MI^{2+})/(M^{2+})(I)$$

$$M^{2+} + G^- \rightleftharpoons MG^+ \qquad K_{MG} = (MG^+)/(M^{2+})(G^-)$$

$$M^{2+} + E \rightleftharpoons ME^{2+} \qquad K_{ME} = (ME^{2+})/(M^{2+})(E)$$

Since we are considering one ligand molecule with several binding modes, the total concentration of each site (T_L) is equal to any other, and the metal ion concentration is the same for each mode. The proportional concentrations of metal ion at each binding mode are given by

$$(MI^{2+}) \sim K_{MI}K_I/[(H^+) + K_I] \qquad (1)$$

$$(MG^+) \sim K_{MG}K_G/[(H^+) + K_G] \qquad (2)$$

$$(ME^{2+}) \sim K_{ME}K_IK_G/[(H^+)^2 + (H^+)K_I + K_IK_G] \qquad (3)$$

Introduction of $(M^{2+})T_L$ as a product in the numerator on the right side would make equalities of the above proportionalities.

To utilize the above three proportionalities the formation constants, the first term on the right, must be estimated. For the substituted glycine binding site in histidine, $pK_G = 9.2$, which is 0.5 log unit less basic than $pK_a = 9.7$ for glycine, where for copper(II) log $K_1 = 8.1$.[11] By subtracting 0.5 log unit from the last formation constant we estimate log $K_{MG} \simeq 7.6$ for copper(II) binding at the substituted glycine site in histidine.

For imidazole, $pK_a = 7.1$ and for the first formation constant with copper(II), log $K_1 = 4.3$.[13] For β-imidazolepropionic acid, $pK_a = 7.6$ and log $K_1 = 4.6$.[14] The slightly greater formation constant in the latter potentially bidentate ligand reflects a correspondingly greater basicity so that interaction of the carboxylate group is quite weak and the predominant interaction of copper(II) occurs at the imidazole nitrogen in both ligands. For histidine the acid ionization constant

(9) K. Sone and S. Utsuno, *Bull. Chem. Soc. Jap.*, **39**, 1813 (1966); D. P. Graddon and L. Munday, *J. Inorg. Nucl. Chem.*, **23**, 231 (1961); J. Bjerrum, C. J. Ballhausen, and C. K. Jørgensen, *Acta Chem. Scand.*, **8**, 1275 (1954).

(10) R. W. Hay, P. J. Morris, and D. D. Perrin, *Aust. J. Chem.*, 21, 1073 (1968).

(11) "Stability Constants," Special Publication No. 17, The Chemical Society, London, 1964. "Best" values near 25° and moderate ionic strengths are chosen.

(12) A. Albert, *Biochem. J.*, **50**, 690 (1952).

(13) Y. Nozaki, F. R. N. Gurd, R. F. Chen, and J. T. Edsall, *J. Amer. Chem. Soc.*, **79**, 2123 (1957).

(14) A. Chakravorty and F. A. Cotton, *J. Phys. Chem.*, **67**, 2878 (1963).

for the imidazolium group $pK_I = 6.1$, and by comparison with the above two model compounds, we estimate the formation constant for copper(II) binding at the imidazole site as $\log K_{MI} \simeq 3.2$.

For copper(II) binding to histidine *via* two nitrogen atoms, histamine has often been considered as a model. For histamine the pK_a values are 6.1 and 9.9 and $\log K_1 = 9.6$.[15] Comparison with the values of pK_I and pK_G for histidine yields $\log K_{ME} \simeq 8.9$ as an estimated formation constant if copper(II) binds to histidine through an imidazole and amino nitrogen atoms. However, lack of an α substituent in histamine renders nonchelatable rotamers more probable, with the result that it is a poor model for estimating formation constants involving chelation in histidine. A better estimate of $\log K_{ME}$ may be obtained by employing histidinol as a model for bidentate binding *via* a pair of nitrogens in histidine. Unlike histamine both histidinol and histidine are disubstituted at the α-carbon atom. For histidinol we find pK_a values of 5.93 and 8.94 and $\log K_1 = 9.35$ (and $\log K_2 = 6.6$). Comparison with the pK_I and pK_G values of histidine gives $\log K_{ME} \simeq 9.8$ as a better estimate of bidentate copper(II) binding to imidazole and amino nitrogens in histidine. Thus the presence of an additional α substituent increases the formation constant by almost one log unit in this case. The last estimated formation constant is only 0.5 log unit less than the observed value[11] of $\log K_1 = 10.3$ for histidine, suggesting that though the anion serves as a tridentate ligand, the binding of the carboxylate group to copper(II) contributes in only a modest way to the overall stability of the complex.

A comparison of copper(II) binding at the bidentate imidazole and amino nitrogen mode to the bidentate glycine locus may be achieved by evaluating the ratio of eq 3 and 2.

$$\frac{(CuE^{2+})}{(CuG^{+})} = \frac{10^{9.8}10^{-6.1}10^{-9.2}[(H^+) + 10^{-9.2}]}{10^{7.6}10^{-9.2}[(H^+)^2 + (H^+)10^{-6.1} + 10^{-6.1}10^{-9.2}]}$$

In the limit of high pH the ratio becomes $10^{2.2}$ so that of the two bidentate binding modes the glycine one occurs about 0.6% as frequently as the mode through two nitrogen donors. At all pH <7 the value of the above ratio is pH dependent. This result is expected, because at lower pH two protons are displaced for each copper(II) bound in the bidentate nitrogen mode and only one for binding at a glycine locus. Only below pH 5 does binding at the glycine locus become greater than 10% of that at the bidentate nitrogen mode, the above ratio becoming unity at pH 3.9. However, the above ratio is not valid at pH <5 because protonated histidine species $CuHL^{2+}$ were not considered and are known to occur.[16,17]

Three sets of authors propose that histidine in the copper(II) complex $CuHL^{2+}$ is bound *via* an imidazole nitrogen and carboxylate group so that the acid ionization with $pK_a = 3.8$ (at 25°) to give CuL^+ takes place at the ammonium group.[16-18] This interpretation is inconsistent with our equations. It can be shown by an analysis similar to the above, but employing equations for histidine monoprotonated at different sites, that Cu(II) coordination in the imidazole–carboxylate mode with a protonated ammonium group never exceeds 5% of that chelated at the glycine locus with a protonated imidazolium group. (Even allowing for a greater value for K_{MI} that may result from a model compound with an additional α substituent in the imidazole–carboxylate mode, the above ratio should not rise above 20%.) The imidazolium mode becomes more important for other metals ions in the first transition series and should be dominant for Mn(II) coordination to neutral histidine. For Cu(II), more competitive with chelation at the glycine locus with a protonated imidazolium group than the above is chelation at the amino–imidazole nitrogen mode with a protonated carboxylic acid group. As before this ratio is pH dependent with the former locus being favored at the lowest pH values and the latter mode increasing in importance as the pH is increased to 4. Because the carboxylic acid ionization from uncomplexed 2+ charged histidine takes place with $pK_a = 1.9$, the ionization occurring in the 2+ charged histidine–copper(II) complex with $pK_a = 3.8$ appears too high to be a carboxylic acid ionization. We propose that the predominant binding of histidine in $CuHL^{2+}$ is at the glycine locus and the deprotonation occurring with $pK_a = 3.8$ takes place mainly from the imidazolium group. This pK_a is less than that of 5.4 observed from the imidazolium group in the similarly charged uncomplexed histidine methyl ester[19] because deprotonation is promoted by subsequent Cu(II) binding at the imidazole group in the histidinate complex. This proposed structure for the protonated complex in solution has been found by X-ray diffraction in the solid state.[20]

Histidine Dipeptides. The CD and absorption spectra of copper(II) complexes of histidine dipeptides are grouped in vertical columns according to approximate wavelengths of CD extrema in Table III together with values for alanine dipeptides. The first two alanine dipeptides are bound to copper(II) by the amino nitrogen, ionized amide nitrogen, and carboxylate oxygen.[7] The fourth position about the chelate plane is occupied by water in CuL^0 and by OH^- in $CuLOH^-$. Despite the change in CD sign, the similar wavelength and intensity in the absorption spectra supports the suggestion that after amide hydrogen ionization at pH 6.0 to give CuL^0 a structure similar to the above also exists for the two L-His-X dipeptides, so that the imidazole side chain is only weakly coordinated, if at all.[21] The early conclusion from potentiometric titrations that glycyl-L-histidine is bound to copper(II) by the amino nitrogen, ionized amide nitrogen, and imidazole nitrogen has been confirmed by X-ray diffraction.[22,23]

(15) B. L. Michel and A. C. Andrews, *J. Amer. Chem. Soc.*, **77**, 5291 (1955).
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Table III. Visible CD and Absorption Spectra of Dipeptide Complexes of Copper(II)

		nm	Δε	nm	Δε	nm	Δε	nm	ε
L-AlaGly	CuL^0			594	−0.11			636	87
	$CuLOH^-$			594	−0.11			637	80
Gly-L-Ala	CuL^0					650	−0.35	635	90
	$CuLOH^-$	500	−0.15			680	−0.32	636	80
L-HisGly	CuL^0					660	+0.36	621	90
Gly-L-His	CuL^0	500	−0.06	594	+0.12			600	58
L-Ala-L-His	CuL^0	500	−0.05			660	−0.04	600	57
L-Ala-D-His	CuL^0	490	+0.01	590	−0.12			592	67
L-His-L-Ala	CuL^0	488	−0.05	576	+0.06	678	−0.08	608	94
	$CuLOH^-$	488	−0.10	580	+0.30	700	−0.10	617	88
L-Carnosine	$Cu_2L_2^0$			556	−1.64	710	+0.21	604	75
L-Homocarnosine	CuL^0	510	−0.15			630	+0.08		

In solution, water occupies the fourth position about the chelate plane in CuL^0. Similar structures presumably occur in the copper(II) complexes of Ala–His in Table III. Additional base promotes ionization of the pyrrole hydrogen to yield a tetrameric complex with four nitrogen donors about each Cu(II).[24] Three d–d transitions are observed in the visible CD of some of the complexes. Except for those containing D-histidine, the first Cotton effect at less than 350 nm for all of the complexes of Table III is positive.

The highly consistent additivity relation found in other dipeptide chelates of copper(II)[7] applies reasonably well to the histidine dipeptides of Table III. For instance, addition of the CD magnitudes near 500 nm for the complexes of L-AlaGly and Gly-L-His yields −0.06 as the predicted value for L-Ala-L-His, and subtraction of the values for the second from the first gives +0.06 as the predicted value for L-Ala-D-His. The predicted values are in reasonable agreement with the observed values in Table III, where other comparisons are also possible. Numerical values of Δε in Table III are surprisingly small, considering the size and complexity of the ligands, implying extensive cancellations of positive and negative CD components, which preclude better agreement in the additivity comparisons. Nonetheless the agreement indicates that optical activity in the visible region is nearly an additive function of independent contributions from amino and carboxyl terminal amino acid residues in the copper(II) dipeptide chelates of histidine as found previously for other dipeptide side chains.[7] The additivity relationship has also been observed in tetragonal dipeptide and tripeptide chelates of palladium(II)[25] and tripeptide chelates of nickel(II).[26]

L-Carnosine (β-alanyl-L-histidine) exhibits the largest CD magnitude in Table III consistent with the unique dimer structure found by X-ray diffraction for this copper(II) complex. The chelate plane about each copper(II) is occupied by an amino nitrogen, an ionized amide nitrogen, and a carboxylate of the ligand considered as a substituted β-alanylglycine and the 3-nitrogen of the imidazole ring from the second carnosine molecule.[27] The identity of the titration curves[28] of copper(II) and either L-carnosine or L-anserine (β-alanyl-L-1-methylhistidine) is most easily accommodated by postulating that the same dimer structure found in the solid complex exists also for both ligands in solution. From the increase of CD magnitudes at 556 nm with an increase in concentration of neutral solutions containing equimolar amounts of L-carnosine and Cu(II), the equilibrium constant for dimer formation is calculated to be about $\log K \sim 3.6\ M^{-1}$. This value is within the range of successive formation constants for complexation of imidazole by Cu(II).[13] Dimer formation in equimolar solutions is virtually complete at concentrations $>5 \times 10^{-3}\ M$. Addition of excess neutral carnosine to a solution containing the dimer structure causes no changes in the visible absorption and CD spectra and at 340, 290, and 260 nm, where $\Delta\epsilon = +0.1, -1.0$, and $+1.8$, respectively. L-Carnosine is the only L-histidine-containing compound to yield a copper(II) complex with a positive Cotton effect near 220 nm. Titration results also indicate that the additional carnosine is unbound and the dimer structure preserved. In contrast, addition of 1 equiv of neutral glycylhistidine to a solution containing the CuL^0 species decreases the absorption wavelength from 600 to 580 nm indicating that more nitrogens are bound to copper(II) on addition of glycylhistidine to a complex containing water in one planar coordination position. The low CD magnitudes observed for the copper(II) complex of L-homocarnosine (γ-aminobutyryl-L-histidine) suggest that the carnosine-type dimer structure is not formed in this case.

Discussion

All the physical methods employed—ultraviolet charge-transfer spectra, visible circular dichroism and absorption spectra, titrations, and formation constants—indicate that the copper(II) complexes of lysine are bound through carboxylate oxygen and α-amino donor atoms with the ε-amino group unbound in positive or uncharged forms. A similar conclusion may be drawn for ornithine complexes with zero net ligand charge and with some qualifications for the anionic ligand. Titration studies as well as the rapidity of its reaction with benzoyl chloride and other reagents indicate that the δ-amino group is only weakly bound to copper(II).[29] In contrast, both lines of evidence indicate that the nickel(II) complex of ornithinate

coordination. The constants may be less than those determined at lower ratios where chelation by a ligand molecule might assume more importance.

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anion is tridentate, as the titration curve for removal of the last equivalent is lower on the pH scale than the curve for the copper(II) complex.[12] Furthermore, the δ-amino group in the nickel(II) complex of ornithinate anion is relatively unreactive.[29] For the copper(II) complexes of ornithinate anion, the relatively long wavelength in visible absorption spectra and the positive CD suggest weak apical chelation of the δ-amino group.[9] The weakness of this apical interaction with copper(II) and its presence in only a fraction of the complexed ornithinate anions bring the results of all physical methods to a consistent conclusion.

For the copper(II) complex of 2,3-diaminopropionate anion, evidence from visible spectra and circular dichroism shows that the ligand is bound in the chelate plane through at least two nitrogen donors, while the formation constant indicates a tridentate ligand, implying apical chelation by the carboxylate group. For the neutral 2,3-diaminopropionic acid, visible spectra, circular dichroism, and formation constants all suggest planar chelation by α-amino and carboxylate groups with an unbound β-ammonium group. A similar set of results suggests an identical conclusion for the copper(II) complexes of neutral 2,4-diaminobutyric acid.

The two basically different types of copper(II) complexes, exhibited by lysine and ornithine on one hand and 2,3-diaminopropionate on the other, come together with 2,4-diaminobutyrate, where the results suggest that several species of complexes exist for this anionic ligand. The following observations support this conclusion: ultraviolet charge-transfer spectra indicate some complexes with two nitrogen donors as in 1,3-diaminopropane; the visible spectrum is equivocal but favors a predominance of nitrogen and oxygen donors; the formation constant implies a tridentate ligand; and the circular dichroism exhibits an unusually high magnitude, as does the molar absorptivity. Furthermore the CD magnitudes of the mixed complexes are not half the 2:1 complex, suggesting that the second ligand is not always bound in the same way as the first. A *cis–trans* choice also arises in these systems. The only reasonable conclusion is that we are dealing with a mixture of binding modes and conformations in the copper(II) complexes of 2,4-diaminobutyrate. For the palladium(II) complexes of the same series of α,ω-diaminocarboxylates, the transition among bidentate binding modes occurred at ornithinate, consistent with the relatively greater tetragonality and proclivity of Pd(II) to bind nitrogen.[25]

Though we have attempted to detail the structures of copper(II) complexes of a series of α,ω-diaminocarboxylic acids, it is necessary to appreciate that the properties of a system may often yield a mixture of species where a single one might have been assumed. For instance, in solutions containing 2 mol of net-zero-charged ligand/mol of copper(II), about half of the metal ion is present as CuL^+ if the ligand is diaminopropionic acid and half as $Cu(HL)_2^{2+}$ if the ligand is diaminobutyric acid. For both ligands most of the remaining copper(II) is divided almost evenly between $CuHL^{2+}$ and $CuLHL^+$.[10] Owing to the almost complete disproportionation of $Cu(HL)_2^{2+}$ for diaminopropionic acid, about three times as much free ligand, as H_2L^+, exists in this system. Regardless of the distribution, an average of two N and two O donors are about the copper(II) plane under these conditions. Thus the CD of complexes of both ligands with net zero charge is composed of weighted contributions from several species. Similar complexities occur in histidine complexes.[17]

One of the goals for this research is an attempt to settle the conflicting views on the mode of histidine binding to copper(II). The answers that arise from this study are that the binding modes are pH dependent and that more than one mode exists for each histidine species. The situation is analogous to the several binding modes inferred for 2,4-diaminobutyric acid, which, like histidine, possesses a pair of chelatable nitrogen atoms separated by three carbon atoms. As already concluded in the section on formation constants, neutral histidine is bound to copper(II) predominantly as a substituted glycine with a protonated imidazolium group.

For the copper(II) complex of histidinate anion the near identity of the ultraviolet charge-transfer absorption to that of the histamine rather than the alanine or norvaline complexes indicates chelations by two nitrogen donors. Comparison of formation constants to those of histamine and histidinol suggests primarily two nitrogen donors with a carboxylate interaction to the metal ion. Perhaps the most dramatic evidence against a simple substituted glycine-like bidentate binding mode[30] for histidinate anion is the pronounced positive visible CD observed for complexes of L-histidinate, L-histidinamide, and L-histidinol. The necessarily glycine-like L-1-methylhistidinate complex exhibits an emphatic negative CD like other L-α-amino acids with noncoordinating side chains. The paramount importance of the bidendate nitrogen mode in histidinate anion chelation to copper(II) obtains further support from calorimetric measurements. The heat of formation of the 2:1 histidine complex reported as −21 to −22 kcal/mol is closer to those for the 2:1 complexes of histamine (−24 kcal) and histidine methyl ester (−19 kcal) than to those for phenylalanine (−11 kcal) and imidazolepropionic acid (−9 kcal), where only glycine-like and imidazole, respectively, binding modes are available.[31] Thus the physical methods agree that histidinate anion

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is bound to copper(II) primarily through the bidentate nitrogen mode in the chelate plane. Several lines of evidence such as the formation constant comparison also suggest a weaker apical chelation by the carboxylate group.

The 2:1 histidinate complex displays a small negative CD peak which is amplified in the mixed complex with histamine and disappears in the mixed complex with glycine. These results suggest that the conformation of the histidine molecule is dependent upon the other donor atoms bound to copper(II). With histamine as the other ligand, histidine tends to bind more in a glycine-like mode than with glycine the other ligand, for which chelation of histidine through two nitrogen donors in the chelate plane becomes more important.[32] For the latter mode the difference between the logarithms of first and second formation constants is unusually large, as shown by the values for histamine,[15] histidinol, and histidine methyl ester.[19] A consequence of this difference of nearly three log units is that though the first formation constant of copper(II) with all of the above three ligands is greater than that with glycine, the second formation constant for the last ligand is greater than any of those for the first three. A recent X-ray structure of a mixed histidinate–threoninate copper(II) complex shows three nitrogens and one carboxylate oxygen in the chelate plane with the histidinate carboxylate bound weakly with a distorted geometry in an apical position.[33] The absence of observed stereoselectivity in the 1:2 complexes of copper(II) and L,D-histidine as compared with L-histidine suggests that the second histidinate ligand is bound in only a bidenate mode.[34]

The tendency for the histidinate anion to adopt a conformation complementary to that of other donor groups bound to copper(II) is consistent with entirely independent results on binding studies in mixed ligand systems. The formation constant of a mixed copper(II) complex with histamine and serine (which in this pH region chelates like glycine[35]) is 200 times greater than the statistical value of four expected on the basis of unmixed formation constants in this system.[36] Since the histidinate anion contains within a single ligand both the histamine-like and glycine-like binding modes, it is then to be expected that the second ligand will bind in a way complementary to the first. This tendency toward complementarity may be described as the principle of substantial permissivity, which for histidinate implies a multiplicity of bidentate binding modes in addition to tridentate ones.

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An Investigation of the Effects of Substituents on the Hydrogen-1 and Boron-11 Nuclear Magnetic Resonance Chemical Shifts of Boron-Monosubstituted Borazine Derivatives.[1] Evidence for π-Electron Delocalization in the Borazine Ring

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Received March 4, 1970

Abstract: The effects of the substituents CH_3, $N(CH_3)_2$, OCH_3, F, Cl, and Br on the ^1H nmr, ^{11}B nmr, and infrared and mass spectral properties of a series B-monosubstituted borazine derivatives ($H_2XB_3N_3H_3$) have been examined in detail. The ^1H and ^{11}B chemical shift data are consistent with the hypothesis that the π electrons of borazine are delocalized, at least partially, and the substituents interact with this π system by means of a resonance effect to alter the π-electron density at the *ortho* and *para* positions. This hypothesis is supported by the similarities between analogous borazine and benzene nmr data, as well as excellent correlations between the borazine ^1H nmr data and ^{13}C nmr and reactivity parameter, σ_R^0, data. These data are recognized to be related to changes in the π-electron density in the benzene ring. The infrared and mass spectral properties were also compared but no trends in the data, consistent with a change in substituent, were discernible. The new compound $H_2FB_3N_3H_3$ was prepared by allowing $H_2(CH_3)_2NB_3N_3H_3$ to react with $BF_3 \cdot O(C_2H_5)_2$. The synthesis of $H_2CH_3OB_3N_3H_3$ from $H_2ClB_3N_3H_3$, CH_3OH, and $N(CH_3)_3$ in pentane solution also represents a new reaction in borazine chemistry.

Borazine is isoelectronic with benzene. All of the chemistry of benzene is consistent with the hypothesis that the π electrons are completely delocalized. However, the situation is very different in the case of borazine. Molecular orbital calculations[2] suggest that the π electrons are partially delocalized, but there are no experimental data which can be conclusively interpreted in terms of either the existence of, or the

(1) Presented at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969.

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