Coordinating Properties of the Amide Bond. Stability and Structure of Metal Ion Complexes of Peptides and Related Ligands

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Contents

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Bruce Martin is a Professor (1965) of Chemistry at the University of Virginia, where he joined the faculty in 1959. He received his Ph.D. degree in 1953 for research on gas-phase photochemistry with the late W. A. Noyes, Jr. Dr. Martin performed postdoctoral research at both Caltech and Harvard, and has been on leave from Virginia at Oxford University. In 1958 in the Harvard laboratory of John T. Edsall he began investigations of metal ion interactions with the amide bond. Dr. Martin's research has been concerned with mechanisms of acyl transfer and enzyme reactions, circular dichroic properties of both the disulfide bond and tetragonal transition metal ion complexes, metal–ion interactions with amino acids, peptides, proteins, nucleosides, and nucleotides, multinuclear NMR of these systems and lipid bilayers, and Tb^{3+} luminescence as a probe of Ca^{2+} sites in proteins and viruses.

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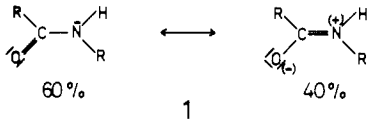
I. Introduction

Amide bonds or groups provide the linkage between adjacent amino acid residues in proteins. When condensation of two amino acids yields a dipeptide, the resulting amide bond is often referred to as a peptide bond (or group). A protein composed of a chain of n amino acids contains $n - 1$ peptide (amide) bonds in the backbone. In aqueous systems hydrolysis of proteins to the constituent amino acids is thermodynamically favored. For this reason peptide bonds in living systems are formed by condensation of an amino group of one amino acid with an activated ester of another with elimination of an alcohol (the ribose of tRNA). The ester function of the (aminoacyl)-tRNA exhibits a higher group-transfer potential (is activated) than ordinary esters under biological conditions, primarily owing to ionization of the more acidic carboxylic acid groups in amino acids (pK_a values of 2.5 for glycine and 4.7 for acetic acid).

The tetrahedral amino nitrogen in an amino acid with $pK_a \simeq 9.7$ loses its basicity upon reaction to give a trigonal nitrogen in an amide bond. Amide groups are planar due to 40% double-bond character in the carbon–nitrogen bond, and the trans form is strongly favored (1).[1] The C–N and C–O bonds possess compa-



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rable amounts of single and double bond character. More recent molecular orbital calculations agree well with the above description (section IIB).

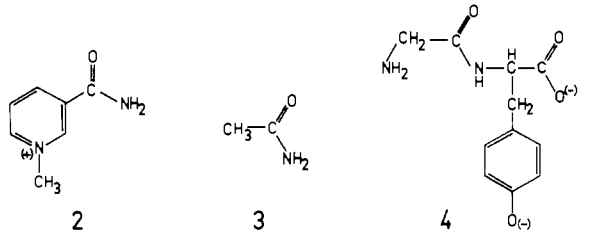
Even before the recognition of the primary structure of a protein as a homogeneous linear chain polymer of defined length and amino acid sequence, one test for proteins used the biuret color produced by the reaction of Cu^{2+} with peptide bonds in alkaline solutions (section IIID). Greater appreciation of protein structure has increased interest in the metal ion binding capabilities of the amide group, so abundant in nature.

An amide group offers two potential binding atoms, the oxygen and nitrogen, for complexation of protons and metal ions. This article describes the stability and structure of metal ions with the amide group, especially as it occurs in peptides and proteins. Without pretending to be exhaustive, this review attempts a comprehensive and critical summary of our current understanding in this area. Also described are optical properties of amide group metal ion complexes, as revealed by both absorption and circular dichroism (optical activity) spectroscopy. Included are aspects of kinetics and mechanism. We write this review now because knowledge of metal ion interactions with the amide group has expanded so rapidly in the last 25 years that our understanding may have reached a plateau.

II. Unidentate Binding

A. Deprotonation

Throughout most of the pH range, in the absence of metal ions the amide group is neutral. It is only a very weak acid for proton loss from the trigonal nitrogen to give a negatively charged species. This very weak acidity makes quantitative equilibrium measurements difficult, and few values appear in the literature. Two separate studies agree that for loss of an amide hydrogen from the aromatic N^1-methylnicotinamide cation (2), $pK_a = 13.3 \pm 0.1$.[2] For aliphatic, neutral acetamide (3) it has been reported that $pK_a = 15.1$.[3] A similar pK_a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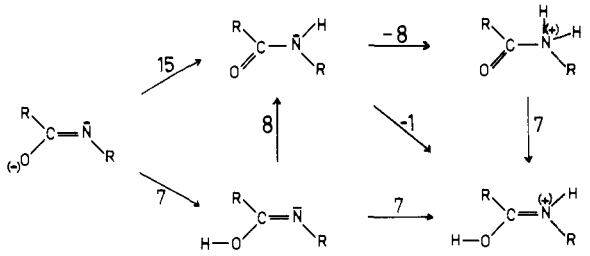


Figure 1. Protonic equilibria in the amide group with representative logarithm of protonation constants located near horizontal and diagonal arrows, and log of tautomeric equilibrium constants in favored direction indicated on vertical arrows. The major species in basic solutions is at left, in neutral solutions at top center, and in strongly acid solutions at bottom right.

of 15.2 has been measured for the glycyltyrosinate dianion (4).[4]

Due to the difficulties encountered in equilibrium measurements, an indirect kinetic method has been used to estimate amide bond acidity. The method rests on the observed hydroxide ion catalyzed amide hydrogen exchange rate and its comparison with the rate expected for an encounter-controlled reaction. For the kinetic method sample reported pK_a values are *N*-methylacetamide, 17.7,[5a] and glycylglycinate, 16.9.[6] However, these estimated values seem high. By allowing for the fact that the equilibrium acidity constants for amides with $pK_a > 13$ are controlled by the encounter-limited rate constant for reprotonation of the amide anion by water (taken as 10^9 s^{-1} M^{-1}), we derive $pK_a = 23 - \log k$, where k is the specific second-order rate constant in s^{-1} M^{-1} for hydroxide ion catalyzed amide hydrogen exchange.[7] Application of this formulation suggests lower pK_a values of 16.4 for *N*-methylacetamide, 14.1 for glycylglycinate, and a value of 15.1 for the peptide bonds in *N*-acetylglycine *N*-methylamide and triglycine.[5] These values agree better with those estimated from equilibrium measurements. In the following discussion we take $pK_a = 15$ for the prototypic amide group in which we are most interested.

B. Protonation

The amide group is also a very weak base, for *N*-methylacetamide $pK_a = -0.7$ or $K_a = 5$.[8] The site of protonation, whether at the amide oxygen or nitrogen, once aroused considerable dispute. Earlier interpretations of infrared results that indicated nitrogen as the protonation site have been revised[9] to allow for the coupled group frequency of the similar C–O and C–N bonds in amides. Nuclear magnetic resonance experiments have established decisively that the oxygen serves as the main protonation site over the entire range of acid solutions from strong[10] to weak.[11,12] The predominance of O-protonation agrees with some degree of negative charge on the oxygen, as indicated in structure **1**, and with results of molecular orbital calculations.[13] Several investigators have estimated the ratio of O- to N-protonated amide cations as 10^7, and for protonation at an amide nitrogen $pK_a = -8$.[5a,11,14]

Though it occurs only 10^{-7} times as frequently as the O-protonated cation, the N-protonated amide cation with a tetrahedral nitrogen may exhibit a correspondingly greater reactivity and serve as the intermediate in some reactions. For example, acid-catalyzed exchange of the nitrogen bound hydrogens in an amide group may proceed by N-protonation to give a small amount of a highly reactive cationic species with a tetrahedral nitrogen[15] or by loss of a nitrogen bound hydrogen from the strongly favored O-protonated cation to give an intermediate tautomer of the neutral amide.[11] The choice has generated lengthy discussions of the likelihood of the two pathways for acid-catalyzed hydrogen exchange.[16] Probably both mechanisms play a role, each predominating for some amides. On the other hand, acid-catalyzed hydrolysis of amides proceeds overwhelmingly via the O-protonated cation, with an insignificant contribution from the N-protonated cation.[17]

C. Deprotonation and Protonation Summary

Figure 1 summarizes conclusions of the proton equilibrium studies. Approximate equilibrium constant logarithms for protonation appear near the arrows. Beginning on the left, the *deprotonated* amide anion protonates at the nitrogen with $\log K_a = 15$ to give the predominant tautomer of a neutral amide at top center of Figure 1. In acid solutions O-protonation occurs with $\log K_a = -1$ and N-protonation with $\log K_a = -8$; these cationic species occur at the right of Figure 1. The number 7 on the descending right-hand vertical arrow indicates that the O-protonated cation is 10^7 times the concentration of the N-protonated cation. For the *N*-methylacetamide O-protonated cation it has been estimated that $pK_a = 7$ for deprotonation at nitrogen to give the rare neutral amide tautomer at bottom center of Figure 1.[11] The 8 on the ascending vertical arrow indicates that the normal neutral amide tautomer is 10^8 times the concentration of the rare tautomer. Values for other equilibrium constant logarithms are derived from properties of a cyclic system.

D. Metal Ion Complexation

The previous discussion on amide group protonation anticipates the results for metal ion complexation. When the amide is anionic, as on the left of Figure 1, protonation and metalation occur at the nitrogen. Just as protonation of a neutral amide (top center of Figure 1) occurs at the amide oxygen (bottom right of Figure 1), so does complexation of most metal ions. The presentation of predominant resonance forms in Figure 1 emphasizes a significant change upon protonation or complexation at a neutral amide: the C–O bond becomes longer and weaker and the C–N bond becomes shorter and stronger (eq 1).

(1)

Both experimental and theoretical results support the increased double bond character in the C–N bond upon protonation and metalation at the amide oxygen. Alkali and alkaline earth metal ions increase the coalescence temperature for observed methyl group magnetic equivalence in the NMR spectra of *N,N*-dimethylamides.[18] Metalation at an amide oxygen increases the double bond character and the barrier to rotation about the C–N bond. This statement rests on results of several theoretical studies of metal ion complexation at the amide group.[19-21] Since binding of metal ions at amide

oxygens increases the barrier to rotation about the C–N bond, this complexation provides no mechanism for protein denaturation due to greater free rotation about the C–N bond.

In contrast, metalation at a neutral amide nitrogen weakens the C–N bond and reduces its barrier to rotation. This conclusion, suggested in Figure 1 for protonation at the amide nitrogen, gains support from a theoretical study.[19] A high concentration of Ag^+ reduces the barrier to rotation about the C–N bond in *N,N*-dimethylacetamide.[22] Only a fraction of the bound, relatively covalent Ag^+ must bind at the amide nitrogen to yield a complex with a tetrahedral nitrogen and free rotation about the C–N bond in order to account for the experimental observation.

There is no conflict between protonation and metalation of the amide oxygen strengthening the C–N bond and at the same time increasing the rate of amide group hydrolysis.[23] Addition of a positive charge at the amide oxygen polarizes the C–O bond, increases the partial positive charge on the carbon, and renders the carbon more susceptible to nucleophilic attack.[21] As mentioned at the end of section IIB, acid-catalyzed hydrolysis proceeds overwhelmingly via the O-protonated amide.[17] In acid-catalyzed amide group hydrolysis, water is the attacking nucleophile while with metalation at the amide oxygen both water and hydroxide ion serve as potential nucleophiles. Metal ions promote amide group hydrolysis in neutral solutions because they introduce a positive charge at the amide oxygen under conditions where the much more nucleophilic hydroxide ion exists in significant concentrations.

With such a weakly basic amide oxygen atom ($pK_a \simeq -1$) strong metal ion coordination will not occur at that site. In aqueous solutions water provides a potent competitive oxygen donor. On the other hand, substitution of a nitrogen bound hydrogen by a metal ion should create a strong bond, but the very weak acidity of this hydrogen ($pK_a \simeq 15$) implies that alkali and alkaline earth metal ions will not effect its removal. Transition-metal ions promise to be more effective in substituting for a nitrogen bound amide hydrogen, but they suffer competition with metal ion hydrolysis and precipitation in neutral and basic solutions. Thus, simple amides in their neutral state form adducts with metal ions at the oxygen, but substitution of an amide hydrogen does not occur in aqueous solutions.[24] With acetamide, potentiometric pH titrations with a 20-fold excess over 2.4 mM Cu^{2+}/2,2′-bipyridyl (to solubilize the metal ion) give no sign of proton release indicative of coordination at the amide nitrogen.[25] Crystal structures of $CdCl_2$ with formamide[26] and of Li^+ with *N*-methylacetamide[27] show only amide oxygen–metal ion interactions.

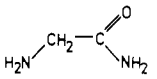
These observations on the weak coordinating capabilities of the "unconnected" amide group point up the necessity for chelate ring formation to bring out its full metal ion binding capabilities. A metal ion needs a primary ligating site or anchor in order to chelate to the amide oxygen and, by substitution of a hydrogen, to the amide nitrogen. By providing an anchor for the metal ion and the possibility of chelation with the amide bond, the primary binding site reduces the importance of metal ion hydrolysis and permits attainment of pH regions where substitution of a metal ion for an amide hydrogen may occur.

We will discuss first the effectiveness of several single primary ligating groups as anchors in promoting chelation with an amide group and keeping metal ions in solution. Then we will proceed to systems with several ligating groups permitting two or more chelate rings. This article emphasizes ligands that are effective in promoting metal ion substitution for a nitrogen bound amide hydrogen.

III. Ligands with a Single Primary Ligating Group

A. Acetylglycinate and Glycinamide

The two terminal groups of peptides and proteins differ markedly in their serviceability as primary ligating groups or anchors for metal ion interactions with an amide group. The simplest model compounds are glycinamide (**5**) for the amino terminus and *N*-acetylglycinate (**6**) for the carboxylate end.



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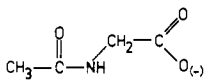

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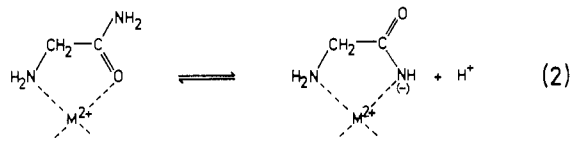
N-Acetylglycinate acts only as a unidentate ligand through the carboxylate group. The ligand is a weak acid with $pK_a = 3.4$, and the stability constant for the Cu^{2+} complex is low, $\log K^{Cu}_{CuL} = 1.3$, and even smaller for other metal ions.[28] The stability constants resemble those of the necessarily unidentate formate ion of comparable basicity.[28] There is no hint of amide hydrogen deprotonation before metal hydroxide precipitation. Crystal structures of the Cu^{2+} complexes show only unidentate coordination via the carboxylate group.[29] Potentiometric pH titration of the ternary systems Cu^{2+}/2,2′-bipyridyl (each 2.4 mM) and *N*-acetylglycinate (48 mM) also failed to reveal any evidence of amide hydrogen deprotonation.[25] In support of this conclusion, in the crystal of the 1,10-phenanthroline–Cu^{2+} complex both of the *N*-acetylglycinate molecules complex only through a carboxylate oxygen; the amide group is not involved in coordination.[30]

Glycinamide chelates metal ions weakly via the amino nitrogen and carbonyl oxygen (**7**), but a stronger chelation occurs upon substitution of an amide nitrogen bound hydrogen by some metal ions such as Cu^{2+} and Ni^{2+} (**8**).



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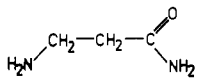
Reaction 2 occurs in neutral solutions with Cu^{2+} ($pK_a = 7.0$)[31] and in more basic solutions with Ni^{2+} (section IVB). A crystal structure of the bis Ni^{2+} chelate of prolinamide anion shows a structure analogous to **8**.[32] In the mixed ligand or ternary complex[33-36] with Cu^{2+} and 2,2′-bipyridyl, the above reaction occurs with $pK_a = 7.7$.[31] However, if the 5-membered chelating ligand glycinamide is extended to the corresponding 6-membered ligand, β-alaninamide (**9**), neither in the binary nor in the ternary Cu^{2+} complexes (with 2,2′-bipyridyl



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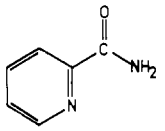
as the second ligand) is amide deprotonation observed,[37] a fact that is in accord with the lower stability of 6-membered chelates compared to 5-membered ones.[33]

The differences in coordination between the amidated and acetylated glycine point up general principles useful for metal ion chelation in peptides. An amino group anchor provides 5-membered ring chelation opportunities at the amide oxygen and nitrogen, both of which occur in glycinamide (5). However, in *N*-acetylglycinate (6), chelation between a carboxylate oxygen and the amide oxygen requires an unlikely 7-membered chelate ring. A favorable 5-membered chelate ring between a carboxylate oxygen and the deprotonated amide nitrogen remains possible, but the weak basicity of the carboxylate group renders it an ineffective anchor for competition with metal ion hydrolysis.

B. Substituted Pyridine Rings

Several pyridine-containing ligands with amide side chains chelate metal ions that promote amide hydrogen ionization. Both Cu^{2+} and Ni^{2+} form chelates with neutral picolinamide (10) through the pyridine nitrogen and amide oxygen. With Cu^{2+} in neutral solutions and



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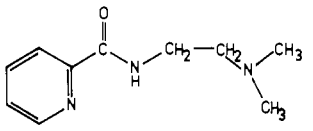
Ni^{2+} in slightly basic solutions one amide hydrogen deprotonates from each of two ligands to form a bis chelate through pyridine and amide nitrogen atoms.[38] Crystal structures of the bis chelate with Ni^{2+} support amide oxygen coordination before and amide nitrogen coordination after amide nitrogen deprotonation.[39]

Amide nitrogen deprotonation also takes place with picolinamide derivatives containing chelating substituents on the amide nitrogen, such as *N*-[2-(dimethylamino)ethyl]-2-pyridinecarboxamide (11), that permit



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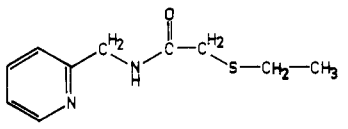
the ligand to become tridentate with Pd^{2+}, Cu^{2+}, and in some cases Ni^{2+} but not Co^{2+}.[40] Insertion of a methylene group between the pyridine ring and the amide carbonyl group of 11 yields tridentate ligands that undergo amide nitrogen deprotonation with Pd^{2+} and Cu^{2+}, but not with Ni^{2+} and Co^{2+}.[41] The amide hydrogen in *N*-(2-pyridylmethyl)-2-(ethylthio)acetamide (12) also undergoes metal ion promoted amide



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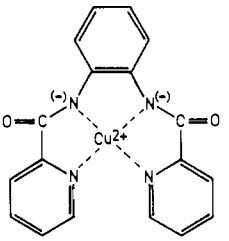
deprotonation.[42] A crystal structure determination reveals that the symmetrical picolinamide derivative, *N,N'*-o-phenylenebis(pyridinecarboxamide) (13), forms



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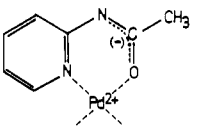
a quadridentate ligand (see also section XVIA) with two pyridine nitrogen and two deprotonated amide nitrogen donor atoms to Cu^{2+}.[43] The decreasing tendency to promote amide deprotonation, $Pd^{2+} > Cu^{2+} > Ni^{2+} > Co^{2+}$, suggested by a comparison among these studies, agrees with the more quantitative results on solutions of peptides reviewed in section IV.

Because the deprotonated amide nitrogen cannot participate in a satisfactory chelate ring in the bis(*N*-(2-pyridyl)acetamide) complex of Pd^{2+}, it has been proposed that instead a 6-membered chelate involves the amide oxygen (14).[44] This structure with a de-



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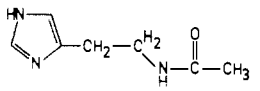
protonated but uncoordinated amide nitrogen has been confirmed in a crystal structure determination.[45] The Co^{2+}, Ni^{2+}, and Zn^{2+} complexes,[45] and possibly also the Cu^{2+} complex,[46] coordinate to the neutral ligand in the same chelate ring, but the amide group remains neutral, with a hydrogen on the nitrogen. When the Pd^{2+} complex[45] with a deprotonated amide nitrogen is compared to the Ni^{2+} and Zn^{2+} complexes with a neutral amide group, the amide C–N bond becomes 0.03 Å shorter with more double bond character while the amide C–O bond becomes 0.04 Å longer with less double bond character. The unusual, deprotonated, uncoordinated amide nitrogen in the Pd^{2+} complex is explained by enforced chelate stereochemistry and weak amide nitrogen basicity.[45] The preference of transition-metal ions to form "intramolecular mixed ligand" species (see ref 35) by coordinating to ligands with heteroaromatic nitrogen residues and oxygen donors may also play a role. However, a deprotonated and uncoordinated amide nitrogen is unlikely to occur with ordinary amides such as those found in peptides.

C. Amino Acid Side Chains

Amino acid side chains may serve as anchors for metal ion chelation with amide groups. For the imidazole side chain of histidine residues in proteins appropriate models with a single primary ligating group are acetylhistamine (15) and imidazole-4-propionamide (16). The latter compound requires an unfavorable 7-membered chelate ring, and its metal ion binding does not seem to have been studied. Cu^{2+} substitutes at the amide nitrogen of the smaller imidazole-4-acetamide in



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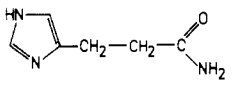

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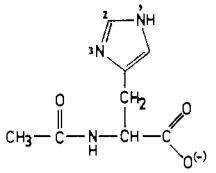
neutral solutions, forming a 6-membered chelate ring.[47] Stability constants for metal ions with imidazole-4-propionate[48] suggest primary complexation at the imidazole ring and little chelation at the carboxylate group.[49]

Acetylhistamine (**15**) requires an unlikely 8-membered ring for imidazole nitrogen–amide oxygen chelation. Substitution of Cu^{2+} for the amide hydrogen to form a 6-membered chelate ring to the amide nitrogen does not occur in neutral solutions of acetylhistamine (**15**) or acetylhistidine (**17**).[47,50] Though accompanied



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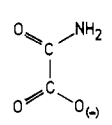
by precipitation, the latter potentially tridentate ligand undergoes amide deprotonation near pH 10 while with acetylhistamine only precipitates have been reported to occur.[47] The apparent inability of acetylhistamine to undergo amide deprotonations has been ascribed to steric hindrance of two ligands about a tetragonal Cu^{2+}.[47] However, acetylhistidine (**17**) presents the same stereochemical problems and Cu^{2+} undergoes easy deformation. With excess ligand at pH 9 a solution of acetylhistamine and Cu^{2+} gives an absorption maximum at 590 nm suggestive of amide deprotonation.[51] The small difference between the two equivocal ligands more probably results from the greater difficulty of deprotonating the appreciably more basic amide nitrogen in acetylhistamine[52] and also perhaps from the tendency of Cu^{2+} to prefer ligand systems with mixed imidazole nitrogen and oxygen donor atoms.[33-36] Acetylhistidine also forms at pH >10 a probable 2:1 tetrahedral complex in which both amide hydrogens have been substituted by Co^{2+}.[53]

These studies confirm that the most favorable chelation of a metal ion bound to a histidyl residue in the interior of a protein is between N-3 of a 4-imidazole side chain and the deprotonated amide nitrogen of the histidyl residue to give a 6-membered chelate ring. If amide nitrogen deprotonation does not occur, a much weaker interaction with the histidyl residue amide oxygen to give a 7-membered ring is possible. Chelation of imidazole side chains with peptide bonds in histidine containing peptides is considered in section XI.

With the possible exception of the phenol (section XVIA1 and XVIB) and the sulfhydryl (section XII) groups, other amino acid side chains have not shown sufficient anchoring capabilities to serve as a single ligating group for metal ion promoted amide hydrogen ionization in peptides and related ligands. The anchoring properties of a single phenol group have to our knowledge only been studied in salicylamide (o-hydroxybenzamide),[47,54] and it appears that in the presence of Cu^{2+} the amide group in this ligand is not ionized.[47] Because most model ligands containing the cysteinyl sulfhydryl side chain have also borne other side chains, this topic is deferred to section XII.

D. Biuret

There exists the possibility that one amide group might serve as an anchor for another. The simplest protein model, acetylglycinamide, does not seem to have been studied, but experience with similar systems suggests that strong chelation with most metal ions does not occur in neutral solutions. Related model compounds are oxalate monoamide (**18**), oxalamide (**19**), and biuret (**20**). From potentiometric pH titrations of



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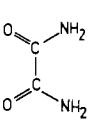

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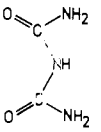

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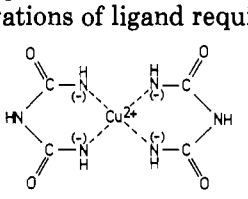
mixed ligand systems consisting of Cu^{2+}/2,2′-bipyridyl (each 2.4 mM) and oxalate monoamide or oxalamide (48 mM) it is evident that in the pH range up to 10 no amide proton is liberated.[25] Similar titrations of Cu^{2+}/biuret and Cu/Bpy/biuret (each reactant was 1.6 mM) gave also no indication[25] for an amide ionization up to pH 9.5. Thus, with these ligands only weak interactions occur in neutral to slightly basic solutions.

In strongly alkaline solutions, however, excess biuret (**20**) with Cu^{2+} forms the well-known violet or biuret color which is the prototype for the complexation of four deprotonated amide nitrogens (from two biuret ligands). The reaction has a long history: it was described in 1848,[55] following up an earlier mention in 1833 that a corresponding reaction occurs with proteins,[56] especially with egg albumin.[57,58] Moreover, at the end of the last century Schiff concluded[57-59] from his systematic studies that the so-called biuret reaction, observed with biuret (**20**) itself, also occurs with malonamide or oxalamide (**19**) and with many of their derivatives. He noted in addition that all those substances which exhibit the biuret color with Cu^{2+} show a yellow color with Ni^{2+}.[57] The correct structure for the alkaline, violet biuret complex of Cu^{2+} was formulated in 1907 as **21**.[60] Investigations of ligand requirements for ap-



21

pearance of a biuret color continued[61,62] and included small glycine peptides.[63,64]

Biuret (**20**) does not deprotonate at pH <11.5,[25] and a value of pK_a = 13.2 has been reported.[65-67] The neutral ligand weakly complexes metal ions via the two oxygen atoms, and some stability constants have been reported in Me_2SO solvent.[68] In crystal structures of complexes of the neutral ligand,[69] $CdCl_4^{2-}$ and $HgCl_4^{2-}$ bind to two unidentate biuret molecules via one oxygen atom,[70] $ZnCl_2$[71] and $CuCl_2$[72] both chelate two biuret molecules via the two oxygen atoms in a 6-membered ring, and Sr^{2+} chelates four biuret molecules via the two oxygen atoms.[73]

TABLE I. Acidity Constants (pK^{H}_{HL}) of Glycine Oligopeptides, Together with Stability Constants (log K^{M}_{ML}) of the Corresponding Cu^{2+} and Ni^{2+} Complexes and pK_a Values for Metal Ion Promoted Peptide Hydrogen Deprotonations[a]

	glycinamide	diglycine	diglycinamide	triglycine	tetraglycine
pK^{H}_{HL}	8.04	8.15	7.91	7.96	7.97
log K^{Cu}_{CuL} (eq 3)	5.40/4.16[b,c]	5.55	5.05	5.24	5.08
pK_1 (eq 4)	7.01	3.99	5.10	5.22	5.50
pK_2 (eq 5)	8.12[b,c]	9.3[d,e]	7.29	6.60	6.89
pK_3			9.8[c,d]	11.9[e,f]	9.29
log K^{Ni}_{NiL}	3.8[c]/3.1[b,c]	4.0[i]/3.2[b,i]	3.4[c]	3.7[i]	3.65[j]
pK_1	(10.5)[g]	9.35[j]	8.5[c]	8.8[l]	(9.1)[g,m]
pK_2	(8.5)[b,g,h]	9.95[b,j]	9.3[c,k]	7.7[l]	(7.1)[g,m]
pK_3			10.5[c,k]	12.8[f,l]	8.2[j]

[a] All values refer to 25 °C and 0.1-0.16 ionic strength. Values for pK^{H}_{HL} and the Cu^{2+} complexes are from ref 80 and 81. [b] Refers to second ligand bound. [c] Reference 82. [d] Coordinated water deprotonation. [e] Reference 83. [f] Displacement of coordinated carboxylate by hydroxide. [g] Species present to a negligible extent; see text, section IVB [h] The sum of pK_1 + pK_2 is from ref 82. [i] References 84 and 85. [j] Reference 84. [k] Both pK_2 and pK_3 have been assigned to coordinated water; see text, section IVB. [l] Reference 86. [m] The sum of pK_1 + pK_2 is from ref 84.

In strongly alkaline solutions biuret loses protons from both terminal nitrogens and they form strong donor atoms in a 6-membered chelate ring. The bis complex with Cu^{2+} (**21**) has a violet or biuret color and the bis complex with Ni^{2+} is yellow. These signifying features have been used in a spectrophotometric method to estimate the product, β_2, of the first and second stability constants: for $Cu(biuret)_2^{2-}$, log β_2 = 22.8; and for $Ni(biuret)_2^{2-}$, log β_2 = 19.6.[74] Spectral features of biuret complexes are discussed in section IXA. For the violet Cu^{2+} complex of biuret, precipitated from strongly alkaline solutions, a crystal structure determination confirms structure **21** with four deprotonated amide nitrogens on two biuret ligands chelated to a tetragonal Cu^{2+}.[75] Similarly, *N*-(hydroxyethyl)-substituted oxalamide forms with Ni^{2+} (again in strongly alkaline solution) a 2:1 complex; the crystal structure analysis[76] of this complex shows square-planar Ni^{2+} coordinated also to four ionized amide nitrogens of two oxalamide ligands. Other oxalamide ligands are discussed in section XVIA2.

E. Summary

To coordinate strongly to the amide group, metal ions must be capable of substituting for a nitrogen bound amide hydrogen. To do so in neutral solutions, metal ions require an effective anchor to inhibit metal ion hydrolysis. The single-ligating groups that serve as effective anchors in neutral solutions can all form 5-membered chelate rings between the deprotonated amide nitrogen and another nitrogen on picolinamide (**10**) and glycinamide (**5**) and their derivatives. Only the glycinamide type ligands model for the terminal amino group in proteins.

The discussion of the two model imidazole side chain ligands (**15**, **16**) suggests that this side chain provides an anchor for chelation only with the amide nitrogen of the same histidine residue in a 6-membered chelate ring. To function as an anchor with its amidated carboxylic acid group, an imidazole group of a histidine residue would have to participate in an unlikely 7-membered chelate ring. Chelation of imidazole side chains with peptide bonds in histidine-containing peptides is considered in section XI.

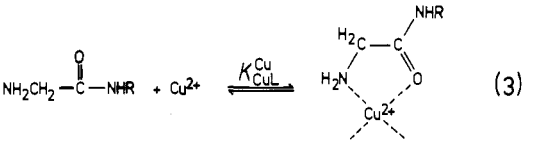
Other ligands such as biuret do not undergo amide hydrogen substitution by metal ions in neutral solutions but do so in strongly basic solutions. The biuret test for proteins conducted in alkaline solutions with Cu^{2+} exemplifies this type of interaction that also occurs with polypeptides, as discussed in section XIII.

IV. Glycine Oligopeptide Complexes

A. Cu^{2+}

1. Stability of Complexes

Twenty-five years ago, on the basis of potentiometric investigations, the coodination of Cu^{2+} with glycyl peptides was correctly formulated.[77,78] As with glycinamide in section IIIA, Cu^{2+} anchors at the amino terminus and chelates to the peptide oxygen of the amino terminal residue as shown in structure **22**. In agree-

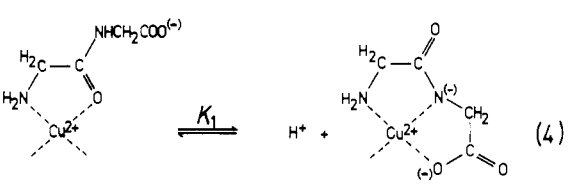


(3)

22

ment with **22**, the properties of the Cu^{2+}–acetylglycylglycine system[79] differ from those of the Cu^{2+}–glycylglycine complex. The stability constant for reaction 3 is K^{Cu}_{CuL}, and the logarithm of this constant for several glycyl peptides appears in the second row of the upper half of Table I, after the amino group pK_a in the first row. What little variation occurs in K^{Cu}_{CuL} parallels the amino group basicity, pK^{H}_{HL}, in accord with structure **22**, a common feature of all 5 peptides in Table I.[80-86]

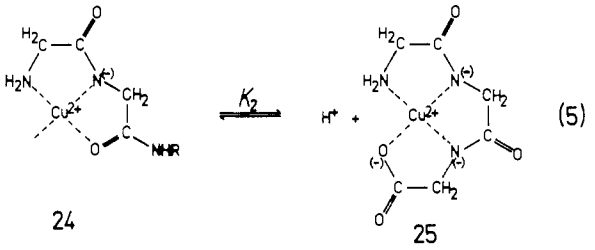
Distinctions among the peptides of Table I become apparent upon substitution of the peptide hydrogen by Cu^{2+} (eq 4), e.g., for glycylglycine (**23**). This depro-



22 23 (4)

tonation fails to occur with glycylsarcosine (glycyl-*N*-methylglycine), demonstrating that a proton disappears from the peptide nitrogen and not from the coordination sphere of the metal ion.[78,83] As seen from the

acidity constants, pK_1, tabulated in the third row of Table I, the substitution reaction 4 occurs with pK_1 = 4.0–7.0, depending upon the ligand. After Cu^{2+} substitution for a peptide hydrogen, glycinamide is only bidentate (structure 8) and exhibits the highest pK_1 of 7.0, while tridentate diglycine with a negatively charged carboxylate oxygen in the third position (**23**) yields the lowest pK_1 of 4.0. The three other peptides in Table I display similar values for pK_1 of 5.1–5.5 due to formation of a tridentate ligand with a neutral amide oxygen in the third position (**24**).

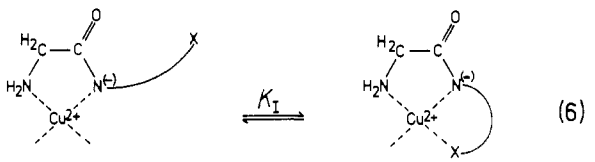


24 25 (5)

For the longer peptides the process continues with Cu^{2+} complexation at the second peptide oxygen as indicated (**24**) passing then to the peptide nitrogen at increasingly higher pH values (eq 5). For example, for triglycine the deprotonation referred to as pK_2 in Table I corresponds to the above substitution reaction 5. Thus for the final triglycine complex (**25**) all four tetragonal positions about Cu^{2+} are occupied by the quadridentate ligand. In the final tetraglycine complex a third peptide nitrogen deprotonates (pK_3 in Table I) and four nitrogens, one amino and three deprotonated peptide nitrogens, occupy the four tetragonal positions about Cu^{2+}.

2. Intramolecular Equilibria and Influence of Ring Size

These conclusions about the promoting effect of an additional binding site on the deprotonation of the amide group may be further elucidated by the recently developed concept about the extent of chelate formation.[87] The participation of the additional ligating group in complex formation may be described by the intramolecular equilibrium 6. The equilibrium con-



open isomer (op) closed isomer (cl) (6)

stant K_I is dimensionless; i.e., the position of this equilibrium is independent of the concentration, but K_I may be calculated[88] with eq 7 provided the experi-

$$K_I = \frac{[Cu(L\text{-}H)_{cl}]}{[Cu(L\text{-}H)_{op}]} = \frac{K_{exptl}}{K_{op}} - 1 \qquad (7)$$

mentally accessible stability constant, K_{exptl}, for the system containing the isomeric mixture has been determined and provided the stability constant of the open isomer, K_{op}, may also be determined or at least estimated.

For the present case of Cu(L-H) of the glycylglycinate system (**23**), we may use the constant measured for the corresponding glycinamide complex (**8**) as the value representing the stability of the open isomer in equilibrium 6. As the acidity constants K^{H}_{HL} of the terminal amino group and the first stability constant K^{Cu}_{CuL} are nearly the same for both ligand systems (Table I), this use is justified. The constants in question, i.e., $pK^{H}_{CuL} = pK_{exptl} = 3.99$ and $pK^{H}_{CuL} = pK_{op} = 7.01$, differ by a factor of 10^3. Hence, for the Cu(glycylglycinate–H) system eq 7 yields $K_I \simeq 1000$, and the closed isomer (**23**) is formed almost exclusively.

The same reasoning for the Cu^{2+}/glycylglycinamide system, based on $pK^{H}_{CuL} = pK_{exptl} = 5.10$ and $pK^{H}_{CuL} = pK_{op} = 7.01$ (Table I), leads to $K_I = 80$. This means, that in this example the closed isomer (with structure **24**) exists "only" to the extent of 98.8% in equilibrium 6. The lower stability of this closed isomer compared with that of the glycylglycinate system reflects the lower coordination tendency of the amide oxygen compared with the carboxylate group, as already noted.

The stability of Cu(glycyl-β-alaninate)$^+$ also agrees with structure **22**, and the corresponding acidity constant for amide ionization ($pK^{H}_{CuL} = 4.67$)[37] reflects the lower stability of 6-membered rings compared with the corresponding value of Cu(glycylglycinate)$^+$ (pK^{H}_{CuL} = 3.99). The additional presence of a carboxylate group in Cu(β-alanylglycinate)$^+$, compared with Cu(β-alaninamide)$^{2+}$ (section IIIA), now allows the ionization of the amide group with $pK^{H}_{CuL} = 4.75$.[37] Cu(β-alanyl-β-alaninate)$^+$, like Cu(β-alanylamide)$^{2+}$, undergoes hydroxo complex formation; i.e., no amide proton is released.[37]

3. An Appreciation of Experimental Difficulties

Reactions 3 and 4 may be abbreviated as

$$L^- + Cu^{2+} \rightleftharpoons Cu(L)^+ \qquad K^{Cu}_{CuL} \qquad (8)$$

$$Cu(L)^+ \rightleftharpoons H^+ + Cu(L\text{-}H) \qquad K_1 (= K^{H}_{CuL}) \qquad (9)$$

to give the sum

$$L^- + Cu^{2+} \rightleftharpoons H^+ + Cu(L\text{-}H) \qquad K^{Cu}_{CuL}K_1 \qquad (10)$$

The product complex of reaction 8 seldom occurs in concentrations greater than 10%; therefore the resolution of the accurately known product $K^{Cu}_{CuL}K_1$ into individual equilibrium constants necessarily introduces error.[89] Ideally one would like to reformulate the reactions as given in eq 11 and 12:

$$L^- \rightleftharpoons H^+ + (L\text{-}H)^{2-} \qquad K^{H}_{L} \qquad (11)$$

$$(L\text{-}H)^{2-} + Cu^{2+} \rightleftharpoons Cu(L\text{-}H) \qquad K^{Cu}_{Cu(L\text{-}H)} \qquad (12)$$

This gives the same sum as above (eq 10):

$$L^- + Cu^{2+} \rightleftharpoons H^+ + Cu(L\text{-}H) \qquad K^{H}_{L}K^{Cu}_{Cu(L\text{-}H)} \qquad (13)$$

Calculation of the stability constant $K^{Cu}_{Cu(L\text{-}H)}$ cannot proceed by the usual techniques because pK^{H}_{L} for peptide hydrogen deprotonation is not accurately known for peptides. An estimate may, however, be provided for the stability constant of a metal ion with a deprotonated peptide ligand if a value for pK^{H}_{L} is assumed. Since the two sum equations (10) and (13) are identical, their associated product equilibrium constants are equal: $K^{Cu}_{CuL}K_1 = K^{H}_{L}K^{Cu}_{Cu(L\text{-}H)}$. The product on the left may be calculated from the con-

stants in Table I, and by assuming $pK^{H}_{L} = 15$ for diglycine (section IIA), we estimate $\log K^{Cu}_{Cu(L-H)} = 16.6$ for Cu^{2+} and dianionic diglycine. The high value for this estimated stability constant results largely from the high value of pK^{H}_{L}. Ignorance of pK^{H}_{L} precludes general application of the second procedure, and despite the difficulty of accurate resolution into K^{Cu}_{CuL} and K_1, the first approach has been generally applied.

4. Crystal Structures

Crystal structure determinations[69] have played an important role in backing the conclusions drawn from potentiometric measurements. Dipeptides that form tridentate chelates to Cu^{2+} through amino nitrogen, deprotonated amide nitrogen, and carboxylate oxygen donors (**23**) include Gly-Gly,[90] Gly-Ala,[91] Gly-Met,[92] Gly-Trp,[93] Leu-Tyr,[94] and Val-Tyr.[95] Two bidentate Gly-Gly molecules chelate to the Cu^{2+} tetragonal plane through amino and deprotonated nitrogen donor atoms.[96]

No deprotonated nitrogens are found in crystal structures of Cu^{2+} complexes displaying a 5-membered chelate ring between the amino nitrogen and the first peptide oxygen (analogous to structure **22**) in the tripeptides Gly-Gly-Gly[97] and Gly-Leu-Tyr.[98] When the peptide hydrogens deprotonate, the amide nitrogens chelate to the Cu^{2+} in Gly-Gly-Gly[99] (structure **25**), Gly-Gly-Gly-Gly[100] (isomorphous to the Ni^{2+} complex shown in Figure 3), and Gly-Gly-Gly-Gly-Gly.[101] In the pentapeptide, deprotonation of the first three peptide nitrogens completes, with the amino nitrogen, the tetragonal plane about Cu^{2+}, and the carboxylate terminal peptide group remains neutral and unbound. The intermediate structure **24** for triglycine, with the amino terminal peptide nitrogen deprotonated and bound to Cu^{2+} and the carboxylate terminal peptide group neutral and bound via the oxygen, has been found in a mixed ligand complex, with imidazole occupying the fourth tetragonal position.[102]

5. Other Structural Studies

Not only the results in Table I but also the sequence of reactions and structures of Cu^{2+}–oligopeptide complexes were derived primarily from potentiometric titrations. As just indicated, crystal structure determinations provide strong support. Visible absorption spectra of metal ion–peptide complexes have been especially informative and warrant separate review in section IXA. In contrast, just as for protonated amides (section IIB), incorrect interpretations of infrared spectra of metalated peptides hindered appreciation of correct structures (section IVF). Other physical methods have been of mixed value.

Calorimetric measurements have been made for several of the reactions occurring with metal ions and peptides. Thermodynamic parameters are available for both Cu^{2+} (cf. ref 103–105) and Ni^{2+} (cf. ref 85) complexes with accompanying interpretations. With dipeptides and Cu^{2+} both reactions 8 and 9 produce an increase in volume.[106]

Electron spin resonance spectroscopy has not been particularly useful and has sometimes been misleading in characterizing Cu^{2+}–peptide complexes. To obtain sufficiently detailed spectra authors often employed frozen solutions and drew conclusions in disagreement with room-temperature-liquid solution studies. However, the lower temperature and concentration of solutes in small pockets magnify the probability of higher order complexes. In many cases application of frozen solution results to liquid solutions has been unjustified. Applications of electron spin resonance spectroscopy to Cu^{2+}–peptide and protein complexes recently received authoritative review.[107]

Mistaken conclusions concerning the structures of oligopeptide complexes have also been made on the basis of selective broadening by paramagnetic Cu^{2+} in ^1H nuclear magnetic resonance spectra. These experiments involve at least a 10^3-fold peptide excess, a condition that produces higher order complexes absent in more nearly stoichiometric ratios. If the Cu^{2+} were tightly bound in a chelate, it would effectively disappear from solution and leave the excess free ligand unperturbed in the NMR spectrum. For selective broadening to occur, the Cu^{2+} must pass rapidly from one ligand to another to produce broadened peaks of all ligand molecules.[108] Unidentate and weak bidentate modes will hasten interligand Cu^{2+} transfer. The rate-limiting process in complex formation of a multidentate peptide with a deprotonated peptide nitrogen includes slow loss of the peptide hydrogen in a ring closure step.[109] At high ligand to Cu^{2+} ratios peptide hydrogen ionization and ring closure do not have time to occur before Cu^{2+} passes onto another ligand. Therefore the peptide-deprotonated chelates are not observable by Cu^{2+} selective broadening.[108,110]

B. Ni^{2+}

Investigations of Ni^{2+} complexes with oligopeptides followed closely on those of Cu^{2+}.[84] While Cu^{2+} forms mainly the tetragonal complexes reviewed in section IVA, Ni^{2+} forms complexes of two distinctly different stereochemistries. Potentiometric titration reveals that by pH 11 deprotonation of the peptide hydrogen in each of two molecules of glycylglycinate has been completed. Stability and acidity constants appear in the lower part of Table I. Glycylsarcosine, in which the peptide hydrogen is replaced by a methyl group, does not show deprotonations corresponding to those of pK_1 and pK_2 of glycylglycine.[84] The resulting solid blue complex of paramagnetic, hexacoordinate Ni^{2+} contains two tridentate Gly-Gly ligands chelated via the amino and deprotonated amide nitrogens and a carboxylate oxygen.[111,112] The result of a recent crystal structure determination appears in Figure 2.

In contrast to diglycine, an equimolar solution of triglycine and Ni^{2+} turns yellow in slightly basic solutions. The pK_1 and pK_2 values[86] of 8.8 and 7.7 (Table I) indicate that the deprotonation of the second peptide nitrogen occurs with a lower pK_a than the first. With glycinamide, triglycine, and higher peptides there is a cooperative transition from a green, paramagnetic, hexacoordinate complex to a yellow, diamagnetic, planar complex.[84] All the ligands in Table I except diglycine form yellow, planar complexes with Ni^{2+}. The two diglycine carboxylate groups preserve the hexacoordinate complex while in the longer peptides one amino and two or more deprotonated peptide nitrogens result in spin pairing to a planar complex. The dia-

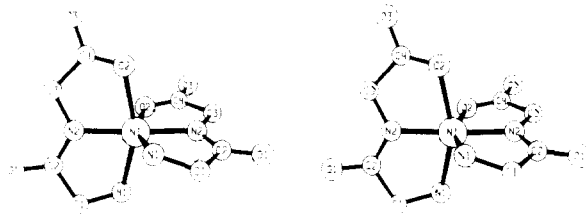


Figure 2. Stereoscopic view of the bis(diglycine) deprotonated peptide nitrogen complex of hexacoordinated Ni^{2+}. Reproduced with permission from ref 112. Copyright 1978, International Union of Crystallography.

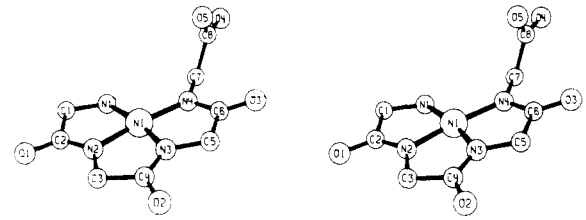


Figure 3. Stereoscopic view of the tetraglycine thrice deprotonated peptide nitrogen complex of planar Ni^{2+}. Reproduced with permission from ref 116. Copyright 1978, International Union of Crystallography.

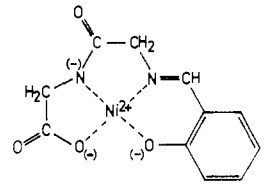
magnetism of the yellow tetraglycine and triglycine complexes becomes evident from their ^1H nuclear magnetic resonance spectra.[113,114] Low concentrations of cyanide reinforce the tendency toward spin pairing in mixed oligopeptide complexes.[115] The result of a crystal structure determination of the planar Ni^{2+} complex of tetraglycine with three deprotonated peptide nitrogens is shown in Figure 3. There are no donor atoms to Ni^{2+} above and below the chelate plane.[111,116] Due to the smaller radius of a spin-paired metal ion, the Ni^{2+} to nitrogen bond lengths are almost 0.2 Å shorter in the diamagnetic tetraglycine complex than in the paramagnetic bis diglycine complex of Figure 2.[111,112,116] In both kinds of Ni^{2+} and Cu^{2+} complexes bond distances to a deprotonated peptide nitrogen are about 0.1 Å shorter than to an amino nitrogen.[69,112,116]

The highly cooperative nature of the transition from hexacoordinate to planar Ni^{2+} complexes of peptides impedes determination of individual acidity constants for peptide deprotonation (pK_1 and pK_2 in Table I). Except for diglycine the titration curves of pH vs. equivalents of added base are almost horizontal in the peptide nitrogen deprotonation region. For glycinamide, triglycine, and tetraglycine, once the first peptide nitrogen is deprotonated the second deprotonation takes place cooperatively with a markedly lower pK_a. The concentration of the species with only one deprotonated peptide nitrogen is very low. In the limit of $K_2/K_1 \rightarrow \infty$ for two peptide nitrogens, the ratio of H^+ concentration at 0.5 and 1.5 equiv is 3, which corresponds to a difference of 0.48 pH unit. To resolve the pK_1 and pK_2 values, the pH difference for the 0.5- and 1.5-equiv points must be greater than 0.5 log unit. For $K_2/K_1 = 100$ this pH difference is 0.50 log unit. Thus the values can be resolved if $pK_1 - pK_2 < 2$. This condition remains unsatisfied for the glycinamide and tetraglycine complexes of Ni^{2+}. At the same time, since notation of only the sum of pK_1 and pK_2 does not suffice to place the titration curve on the pH axis, this procedure loses information.

For the Ni^{2+} complexes of glycinamide and tetraglycine the pK_1 and pK_2 values in Table I are enclosed in parentheses and arbitrarily separated by 2 log units to indicate that they are unresolved and that the difference $pK_1 - pK_2 > 2$. The sum of the two pK_a values is fixed at 19.0 for glycinamide and about 16.2 for tetraglycine. Diglycinamide appears to be a special case in which both pK_3[82] and pK_2[117] have been assigned to deprotonation of coordinated water. It is likely, however, that the titration macroconstants should not be simply assigned to a single group but considered as composites of individual group microconstants[118] for two peptide nitrogen and one coordinated water deprotonations.

Upon comparison of the pK_a values for tetraglycine peptide nitrogen deprotonation in Table I, it becomes evident that pK_3 occurs at lower pH with Ni^{2+} (8.2) than with Cu^{2+} (9.3). The titration curves for the two metal ions with tetraglycine cross so that at pH > 8.3 the Ni^{2+} complex is more stable.[84] This special stability results from the additional ligand field stabilization that occurs in a spin-paired planar Ni^{2+} complex with an amino and three deprotonated peptide nitrogens.

Coordinated dipeptides may form a Schiff base in the coordination sphere without disrupting the whole complex including the ionized amide group. An example is the Ni^{2+} complex of glycylglycinate which forms a Schiff base with salicylaldehyde (**26**).[119] Formation of



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serylglycine but not glycylserine from glycylglycinate and formaldehyde in the presence of an equimolar amount of Cu^{2+} in alkaline solution likely proceeds via an intermediate Schiff base complex formed from glycylglycinate and formaldehyde.[119]

C. Pd^{2+}

Pd^{2+} forms diamagnetic, strongly planar complexes already spin paired. Pd^{2+} complexes with oligopeptides resemble the yellow planar Ni^{2+} complexes discussed above.[120] Substitution of the peptide hydrogen by Pd^{2+} occurs in such acid solutions that the pK_a value is difficult to measure accurately. For triglycine, substitution of the second peptide hydrogen by Pd^{2+} occurs with $pK_a \simeq 4$. Smaller pK_a values appear for the first peptide hydrogen of triglycine and for diglycine.[120] Amide deprotonation in the bidentate complexes formed between $Pd(en)^{2+}$ and glycinamide and diglycine occur with $pK_1 = 2.47$ and 3.76, respectively.[121] Without the chelated 1,2-diaminoethane (en) the latter value for bidentate diglycine should be significantly lower upon formation of a tridentate chelate. We estimate that favorable Pd^{2+}-promoted peptide deprotonations take place with $pK_a \sim 2$. As with the other metal ions, optical properties of Pd^{2+} chelates are reviewed in section IX.

D. Co^{2+} and Co^{3+}

In oxygen-free neutral solutions Co^{2+} forms weak,

pink complexes with glycylglycinate chelated by the amino nitrogen and amide carbonyl oxygen (analogous to structure **22**). Near pH 10 Co^{2+} substitutes for the peptide hydrogen on two ligands to give a light blue bis tridentate complex with a structure similar to that of the Ni^{2+} complex shown in Figure 2. The resulting dianionic light blue complex exhibits an intermediate magnetic susceptibility, and it has been suggested that it exists as an equilibrium mixture of high- and low-spin Co^{2+} complexes differing in bond lengths.[122,123] The light blue color due to an absorption band with a maximum near 610 nm is assigned to a transition within the low-spin component of octahedral Co^{2+}. Deprotonation of the amide hydrogen and coordination of the Co^{2+} at the amide nitrogen appear essential for oxygenation of dipeptide complexes. With glycylsarcosine, in which the amide hydrogen is replaced by a methyl group, Co^{2+} does not produce the light blue color or undergo oxygenation and oxidation to Co^{3+}.[122]

In the presence of O_2 the bis diglycine complex of Co^{2+} with two deprotonated peptide nitrogens forms the corresponding wine–red, anionic Co^{3+} complex with a structure similar to that in Figure 2. The reaction is preceded by formation of a yellow or brown binuclear $[Co^{III}(GlyGly)_2]_2O_2^{4-}$ complex. The binuclear complex may be viewed as an oxygenated complex of Co^{2+} but is best described as a peroxo complex of two Co^{3+} that is in facile equilibrium with free O_2 and the initial mononuclear Co^{2+} complex. The peroxo group occupies one coordination position on each Co^{3+} that has been vacated by a carboxylate of one GlyGly. In a second, irreversible reaction peroxide is released, the carboxylate is replaced, and the mononuclear wine-red Co^{3+} complex analogous to the structure in Figure 2 forms. This structure for the anionic Co^{3+} complex has been confirmed by two independent crystal-structure determinations.[124,125] Complexes with this structure may be prepared by a variety of routes.[126] Triglycine yields Co^{2+} and Co^{3+} complexes similar to those of diglycine with the coordinating groups of the two ligands being amino and deprotonated amide nitrogens and either the carboxylate oxygen of the dipeptide or the carbonyl oxygen of the central glycyl residue of the tripeptide.[127] By a different procedure quadridentate triglycine complexes with the ligand chelated in a plane about hexacoordinate Co^{3+} with amino nitrogen, two deprotonated peptide nitrogens, and a carboxylate oxygen may be prepared.[128]

The preceding outline of the formation of the irreversible, wine-red mononuclear Co^{3+} complex by oxygenation of the Co^{2+} complex does not admit difficulties that have arisen in establishing release of peroxide. Though sought, this product has not been found in the required amounts. It is possible that released peroxide reacts relatively rapidly with ligand, which is in excess; such peroxidase-like reactions are well-known with Cu^{2+}.[129] Extensive investigations have been made of the binuclear oxygenated[130,131] and irreversible Co^{3+} complexes with dipeptides.[126,132-134] In the Co^{3+}–diglycine complexes the carboxylate terminal methylene hydrogens undergo exchange in basic solutions.[126,134,135] In contrast, if the amino group is first incorporated into a Schiff base linkage, the corresponding complex undergoes hydrogen exchange in the amino terminal residue.[119] The amide group is also involved in glycylhistidine complexes of Co^{2+}, the binuclear peroxo–Co^{3+} (oxygenated Co^{2+}) intermediates, and the mononuclear irreversible Co^{3+} products; there are, however, several species in this system.[132,136,137] By having "failed to concentrate on all the molecular species in solution", an attempt[138] to show that mono and bis GlyGly complexes of Co^{2+} react with O_2 is invalid because at the only pH considered (pH 11) peptide deprotonations in the complexes are ignored.

E. Zn^{2+}

Zinc ion occurs frequently in proteins, and there have been a variety of claims that it is capable of substituting for an amide hydrogen in peptides and other small amides. With the glycine oligopeptides, Zn^{2+} complexes mainly to the COO^- with a stability constant, log K, of 0.7 at pH <4, comparable to that found for acetylglycine,[28] indicating comparable modes of interaction (section IIIA).[5b] At pH >4 chelation occurs at the amino terminus and the amide carbonyl oxygen (analogous to structure **22**) with a stability constant, log K, of 3.1,[5b] notably less than the values for Cu^{2+} and Ni^{2+} in Table I, but comparable to that of Co^{2+}. Crystal structure determinations confirm structure **22** for the Zn^{2+} complexes of both glycylglycine[69] and triglycine.[139] In contrast to Co^{2+}, however, there is little evidence to support peptide hydrogen deprotonation in Zn^{2+} complexes of glycine oligopeptides. There are several reasons why peptide deprotonation should be more difficult to observe with Zn^{2+} than with Co^{2+}, with which it occurs near pH 10 with glycine peptides (section IVD). Though both Co^{2+} and Zn^{2+} gain energetically by substitution of N for O donor atoms, the gain is slightly greater for Co^{2+} due to a ligand field stabilization upon substitution of the stronger donor atom. In the case of bis dipeptide complexes there is an additional stabilization for Co^{2+} due to conversion to the low spin state. Taken together we project any Zn^{2+}-promoted glycine oligopeptide deprotonations to occur at pH >10. In this pH region competition from hydroxo complex formation is severe, and Zn^{2+} exhibits a stronger tendency to undergo hydrolysis than Co^{2+}.

Even though Zn^{2+} appears unable to substitute for a glycyl peptide hydrogen, its chelation does facilitate its exchange with solvent protons.[5b] This result is expected for chelate structures such as **22** where metal ion interaction at the amide carbonyl oxygen speeds proton exchange at the nitrogen.

In more complex peptides, Zn^{2+}-promoted peptide deprotonation has been suggested to occur at about pH 11 with glutathione[140] and pH 7 with glycylhistidine.[50,141] The latter ligand should present one of the most favorable cases for Zn^{2+}-promoted peptide hydrogen deprotonation. Oxygen-free solutions of Co^{2+} and glycylhistidine also titrate the peptide hydrogen with $pK_a \simeq 7.2$.[132,137,141] In addition, evidence has been presented for a Zn^{2+}- and Co^{2+}-promoted amide deprotonation in *N*-[(aminocarbonyl)methyl]iminodiacetate.[142]

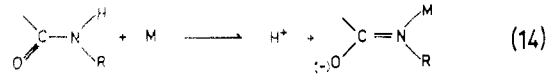
F. Conclusions

Three first-row transition metal ions and Pd^{2+} substitute with facility for a nitrogen-bound amide hydrogen in solution. The order of peptide hydrogen displacement with a representative pK_a value for small peptides given in parentheses is Pd^{2+} (2) > Cu^{2+} (4) >

Ni^{2+} (8) > Co^{2+} (10). Two inert metal ions have also been shown to substitute for peptide protons: Co^{3+} was reviewed in section IVD, and for Pt^{2+} this is evident from the crystal structure of a glycylmethionine complex.[143] Both inert metal ions appear at least as effective as Pd^{2+}.

It has been claimed that Fe^{3+} displaces amide protons from a multidentate chelate at pH <4.[144] The illustrated titration curve shows precipitation at pH <3.5. We reassign the assumed amide protons to formation of hydroxides.

The planarity of the amide group is maintained in the deprotonated trigonal peptide nitrogen complexes of all metal ions studied. Crystal structures reveal that compared to the free or peptide oxygen complexed ligand, complexation of the peptide nitrogen shortens the peptide C–N and lengthens the C–O bond.[69] Table II tabulates representative bond lengths, including those of recent crystal structure determinations, under several sets of conditions.[1,69,145] Upon substitution of an amide proton by a metal ion the bond length changes are in the direction of more double bond character in the C–N bond and less double bond character in the C–O bond (eq 14), but not so much as to cause us to write struc-


(14)

tures of complexes in this way. It is evident from Table II that both the C–O and C–N bonds possess appreciable amounts of double-bond character both before and after substitution of a metal ion for the peptide hydrogen.

The most basic site in the amide group as indicated in Figure 1 is always the one that is protonated or metalated. In a neutral amide the most basic site is the oxygen and protonation (section IIC) and metal ion complexation occur at that atom. After amide deprotonation, the most basic site is the nitrogen atom where either protonation or metal ion complexation takes place. When either protonation or complexation has occurred at an ionized amide nitrogen, the amide oxygen becomes the most basic center. Any additional rapid protonation or complexation then occurs at the oxygen.[123] Thus a crystal structure shows that in an inert acidic Co^{3+} complex of glycylglycine tridentate chelation occurs via amino nitrogen, deprotonated peptide nitrogen, and carboxylate oxygen donors and additional protonation at the peptide oxygen.[124] The peptide nitrogen has never been found to bear both a proton and a metal ion in any crystal structure determination.[69] The metal ion substitutes for the proton at the trigonal peptide nitrogen. The principles just enunciated were originally applied correctly in the early[77,78] and subsequent work on peptide complexes, but there was an unfortunate diversion of proposing structures with metalation at nitrogen atoms of neutral peptide groups.[146] All such structures are incorrect. They were based partly on faulty interpretations of infrared spectra reminiscent of the O- vs. N-protonation of the amide group controversy reviewed in section IIB.

Complexes with deprotonated peptide nitrogens coordinated to metal ions may bind protons at the peptide oxygen. This protonation site has been identified in a structure determination of inert $Co^{III}(GlyGly\text{-}H)_2^-$ where both peptide oxygens are protonated.[124] For this complex the successive acidity constants for peptide oxygen protonation are $pK_a \simeq 1.3$ and ~ 0.0.[126,148] The related Co^{3+} complex $(NH_3)_3Co(GlyGly\text{-}H)^+$ shows $pK_a = 0.4$.[134] For amide-deprotonated peptide complexes of Cu^{2+}, Ni^{2+}, and Pd^{2+}, the most basic $pK_a \simeq 2.4$.[149,150] Greater values of $pK_a = 4.2$ for peptide oxygen protonation occur in complexes of Ni^{2+} and tetraglycine[151] and Cu^{2+} and glycylglycylhistidine.[152] The increased basicity was attributed to protonation at the C-terminal peptide oxygen and internal hydrogen bonding with the unbound carboxylate group. However, the additional factor of about 60 is in accord with that expected for the electrostatic effect of the nearby negative charge.

TABLE II. Bond Lengths (in Å) of C-N and C-O Bonds[a]

conditions	C-N	C-O
double bond	1.24	1.20
peptide N-metal ion	1.30	1.27
free peptide	1.325	1.24
single bond	1.45	1.42

[a] Values collected from ref 1, 69, and 145.

At pH above 11 OH^- drives off the chelated carboxylate in tripeptide complexes (analogous to structure **25**). The pK_a for this displacement increases in the order $Pd^{2+} < Cu^{2+} < Ni^{2+}$. Presence of a side chain in the carboxylate terminal amino acid residue increases the pK_a value.[153]

V. Dipeptides with Weakly Coordinating or Noncoordinating Side Chains

In this section we describe trends in stability and amide hydrogen deprotonation constants in dipeptides containing side chains that are either noncoordinating or exhibit relatively little tendency to coordinate. Included are alkyl side chains with and without hydroxyl and thioether groups. Few studies have dealt with peptides containing aspartyl or glutamyl residues with their carboxylate side chains. Imidazole side chains of histidyl residues are discussed in section XI and sulfhydryl groups of cysteinyl residues in section XII.

A. Gly-X and X-Gly Dipeptides

1. Stability and Structure of $Cu(L)^+$ Complexes

As described in section IVA the initial, low pH complex formed by peptides chelates the metal ion between the terminal amino nitrogen and the amide oxygen of the amino terminal amino acid residue (structure **22**). Hence the nature of the amino terminal residue in a peptide should show the greatest influence on the stability of the N,O-chelated metal ion. Figure 4 shows a plot of log K^{Cu}_{CuL} for formation of the N,O-chelate (see eq 3) vs. pK^{H}_{HL} for ammonium group deprotonation in the free ligand. Results for the dipeptide complexes are tabulated in Table III.[81,154,155]

Since the range of pK^{H}_{HL} for Gly-X dipeptides in Table III is only 0.16 log unit, results for other peptides[80,81] that contain an amino terminal glycyl residue have also been included in Figure 4. All data for the Gly-X dipeptides are solid circles; these fall near the solid straight line with a relatively steep slope of 2.0 ± 0.1. In contrast, X-Gly dipeptides with side chains other than hydrogen in the amino terminal residue, represented by the open circles, deviate from the solid

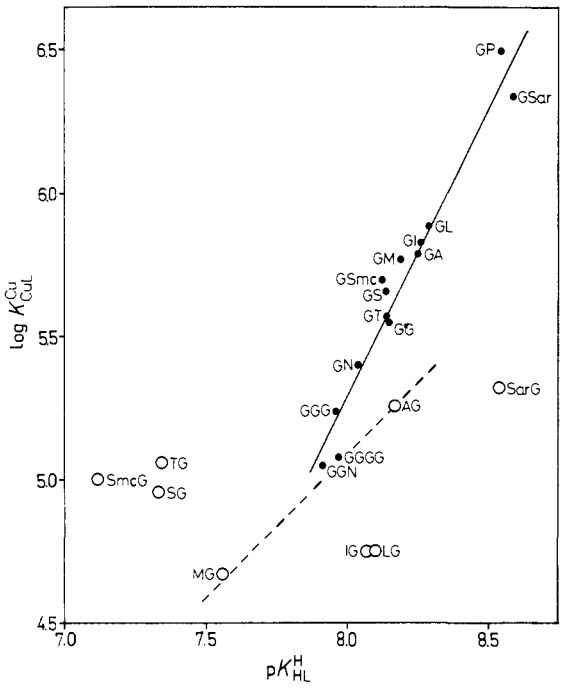


Figure 4. Plot of log stability constant for bidentate Cu^{2+} chelation by an amino nitrogen and amide oxygen of peptides (log K^{Cu}_{CuL}; eq 3) vs. pK for ammonium group deprotonation (pK^{H}_{HL}). Solid circles and solid line of slope (2.0) (standard deviation ±0.1) refer to peptides with an amino terminal glycyl residue. Open circles and dashed line of unit slope drawn through point for Ala-Gly refer to X-Gly dipeptides. For the abbreviations see the first column in Table III; in addition GN = glycinamide, GP = glycyl-L-proline, GSar = glycylsarcosine, GGG = triglycine, GGN = glycylglycylamide, GGGG = tetraglycine, SarG = sarcosylglycine. The data are from ref 81 and 154 (I = 0.1, $NaClO_4$; 25 °C).

line of steep slope and exhibit no apparent regularity. Though a similar plot of Cu^{2+} complexes of amino acids is also irregular, a slope of less than unity has been suggested.[156] In order to provide an additional reference line for the X-Gly dipeptide complexes in Figure 4, a short dashed line of unit slope has been drawn through the point for Ala-Gly. As in the case of the amino acid glycine[156] the point for the Gly-Gly system in Figure 4 falls away from the dashed line of unit slope for X-Gly dipeptides.

Points for Sar-Gly, Leu-Gly, and Ile-Gly in Figure 4 fall below both the solid and dashed lines. These negative deviations may be ascribed to steric hindrance due to the *N*-methyl group of sarcosine and the branched hydrocarbon side chains in the last two dipeptides.[157,158] In contrast, the points for *S*-methylcysteinyl-Gly (SmcG), Ser-Gly, and Thr-Gly fall above the solid and dashed lines. These positive deviations may be attributed to involvement of the sulfur and oxygen atoms of the side chains in apical interactions with Cu^{2+}.[159] The point for Met-Gly falls nearly on the dashed line of unit slope, suggesting either a cancelation of steric and apical interactions or a lack of any to begin with. This observation parallels that made with amino acid complexes of Cu^{2+}: it has been concluded that the ether sulfur atom in *S*-methylcysteine interacts weakly with Cu^{2+} while that of methionine does not.[108,156,162,163]

On the basis of the dashed line of unit slope in Figure 4 and the extent of the positive deviation one may estimate with eq 7 (see section IVA2) the extent of the apical interaction in the Cu^{2+} complexes of Ser-Gly, Thr-Gly, and SmcG; the increased stabilities, log K^{Cu}_{CuL} − log K_{op} (eq 7), are about 0.5, 0.6, and 0.8 log unit, respectively. This corresponds to values of 2, 3, and 5 for the intramolecular equilibrium constant K_I; hence the side chains participate in complex formation of $Cu(SG)^+$, $Cu(TG)^+$, and $Cu(SmcG)^+$ to the extent of 67, 75, and 83%. These estimates are lower limits; calculations based on the solid line with the steep slope would result in somewhat larger percentages. The important implication from these calculations is that there is an intramolecular equilibrium between an open isomer with structure **22** and a "closed" isomer with an apical side chain interaction.

We now summarize this section on the relative stabilities of the initial, NO-chelated Cu^{2+} complexes of dipeptides. In the X-Gly dipeptide complexes those of Leu-Gly and Ile-Gly exhibit reduced stability due to steric hindrance of branched side chains. The complexes of SmcG, Ser-Gly, and Thr-Gly display enhanced stability due to apical involvement of electronegative side-chain atoms. When the order of amino acids in these five dipeptides is reversed to give Gly-X dipeptides, all five yield stability constants close to the straight line for other glycyl-peptide complexes. Because only the Gly part of the Gly-X dipeptides share

TABLE III. Acidity Constants (pK^{H}_{HL}) of Dipeptides and Equilibrium Constant Logarithms of Cu^{2+}-Dipeptide Systems[a]

dipeptide	pK^{H}_{HL}	log K^{Cu}_{CuL}	pK^{H}_{CuL}	log K^{Cu}_{CuL} − pK^{H}_{CuL}
glycyl-L-alanine (GA)	8.25	5.79	4.04	1.75
glycyl-L-leucine (GL)	8.28	5.89	4.76	1.13
glycyl-L-isoleucine (GI)	8.26	5.83	4.71	1.12
glycyl-L-serine (GS)	8.14	5.66	3.77	1.89
glycyl-L-threonine (GT)	8.14	5.57	4.14	1.43
glycyl-*S*-methyl-L-cysteine (GSmc)	8.12	5.70	3.75	1.95
glycyl-L-methionine (GM)	8.19	5.77	3.96	1.81
glycylglycine (GG)	8.15	5.55	3.99	1.56
L-alanylglycine (AG)	8.17	5.26	3.64	1.62
L-leucylglycine (LG)	8.10	4.75	3.26	1.49
L-isoleucylglycine (IG)	8.07	4.75	3.26	1.49
L-serylglycine (SG)	7.33	4.96	3.60	1.36
L-threonylglycine (TG)	7.34	5.06	3.55	1.51
S-methyl-L-cysteinylglycine (SmcG)	7.11	5.00	3.44	1.56
L-methionylglycine (MG)	7.56	4.67	3.19	1.48
L-alanyl-L-alanine	8.17	5.54	3.72	1.82
L-alanyl-D-alanine	8.32	5.71	3.96	1.75
L-leucyl-L-leucine	7.91	5.21	3.88	1.33
L-leucyl-D-leucine	8.20	5.48	4.88	0.60

[a] The last four entries are from ref 155 (I = 0.1, KNO_3; 25 °C); all others are from ref 81 and 154 (I = 0.1, $NaClO_4$; 25 °C).

in N,O-chelation, the side chains of the X part are not involved in steric or apical interactions.

2. Amide-Ionized Cu(L–H) Complexes

At pH ~4 the dipeptides, chelated to Cu^{2+} in a bidentate mode by amino nitrogen and amide carbonyl oxygen atoms, undergo deprotonation and coordination at the amide nitrogen. The resulting terdentate chelate (structure **23**) with a planar peptide bond is notably more rigid than its bidentate precursor (eq 4). The corresponding acidity constants for proton release, pK^{H}_{CuL}, in the fourth column of Table III refer to reaction 9:

$$CuL^{+} \rightleftharpoons Cu(L\text{-}H) + H^{+} \qquad (9)$$

Unusually high values of pK^{H}_{CuL} = 4.76 and 4.71 occur for Gly-Leu and Gly-Ile. This retardation of amide hydrogen deprotonation has been assigned to steric inhibition of peptide hydrogen deprotonation.[81,123,154] The branched side chains inhibit deprotonation of the amide nitrogen on the same amino acid residue. Similar inhibition of peptide hydrogen deprotonation occurs in the Ni^{2+} complexes of Gly-Leu and Gly-Val[84,164] and in the Cu^{2+} complexes of the tripeptides Gly-Leu-Gly and Gly-Gly-Leu.[165]

The stability of the terdentate dipeptide complexes chelated by amino nitrogen, deprotonated peptide nitrogen, and a carboxylate oxygen atom (**23**) may be measured by reaction 10. This reaction sums reactions

$$Cu^{2+} + L^{-} \rightleftharpoons Cu(L\text{-}H) + H^{+} \qquad (10)$$

8 and 9, and its equilibrium constant logarithm is given by $\log K^{Cu}_{CuL} - pK^{H}_{CuL}$. A listing of this difference for dipeptide complexes of Cu^{2+} appears in the fifth column of Table III. The low values for Gly-Leu and Gly-Ile result from steric inhibition of amide deprotonation mentioned above. With Gly-Thr excluded, the other values for the Gly-X dipeptides span the narrow range 1.75–1.95. That the wider variations in the separate constants $\log K^{Cu}_{CuL}$ and pK^{H}_{CuL} are offset in their difference becomes more evident from the X-Gly dipeptide complexes where for all seven complexes the range is from 1.36 to 1.62. The reason for the offsetting effects in the difference $\log K^{Cu}_{CuL} - pK^{H}_{CuL}$ can be seen by considering their component reactions 8 and 9. The first chelation reaction depends on the basicity of the amino terminal residue and the amide group. The second proton loss and rearrangement depend upon the acidity of the amide group. To an appreciable extent these two quantities are influenced in opposite directions by a given side chain substituent on the ligand.

The equilibrium constants obtained in solution indicate no significant interaction of the methionine side chain with Cu^{2+} in the terdentate Cu(L-H) complexes of Gly-Met or Met-Gly. A Cu^{2+}–thioether interaction in Gly-Met and Met-Gly was detected by visible absorption only before peptide hydrogen deprotonation and only at the liquid nitrogen temperature.[166] In support of the solution results, the crystal structures of the Cu^{2+} complexes of Gly-Met[167] and Met-Gly[168] show terdentate chelation of the dipeptide backbone without coordination of the ether sulfur. In contrast, a crystal structure of the class b (soft) Pt^{2+} with terdentate Gly-Met shows chelation by amino and deprotonated amide nitrogens and by the ether sulfur.[143] The coordination tendency of isolated thioether groups has been characterized.[88,169,170]

There are few equilibrium constant results for peptide ligands with other metal ions. Comparison of the relative equilibrium constant values for Co^{2+} complexes of Gly-Gly, Gly-Ser, glycylaspartate, and aspartylglycinate[137] reveal trends similar to those described for Cu^{2+} complexes.

B. Dipeptides with Two Non-Glycyl Residues

The last four entries in Table III list results for Cu^{2+} complexes of two pairs of diastereomeric dipeptides, Ala-Ala and Leu-Leu.[155] In the free dipeptide the main determinant of conformation are nonbonded interactions that place the end groups nearer to each other in L,D- than in L,L-dipeptides and result in a more acidic carboxylic acid group and a less acidic ammonium group in the L,D isomer. There is little change in conformation upon going from cationic to zwitterionic to anionic dipeptide; Coulombic attractions between oppositely charged end groups in the zwitterion affect the conformation only slightly.[171,172]

For the $Cu(L)^{+}$ complexes the L,D or mixed isomer is more stable than the L,L or pure isomer. This greater stability results from a greater basicity of the amino group. For each L,L and L,D pair the difference $\log K^{Cu}_{CuL} - pK^{H}_{HL}$ is nearly identical: -2.62 ± 0.01 for the alanine pair and -2.71 ± 0.01 for the leucine pair (Table III). This result implies a slope of unity in a plot analogous to Figure 4 for each pair. Thus, the stability constants are proportional to the acidity constants, and the equilibrium constant product $K^{Cu}_{CuL}K^{H}_{HL}$ for the reaction of Cu^{2+} with the protonated ammonium ligand (eq 15) is nearly identical for each diastereomeric pair.

$$Cu^{2+} + HL \rightleftharpoons CuL^{+} + H^{+} \qquad (15)$$

The 0.1 log unit greater value for the Ala over the Leu pair may be ascribed to greater steric hindrance in the latter pair. For the alanine pair the difference of –2.62 is nearly equal to that for glycylglycine (–2.60). It is curious that though the constants for reaction 15 are nearly equal for each diastereomeric pair, they differ systematically from those for the related glycyl dipeptides with a single side chain. The difference of the logarithm of the constants $\log K^{Cu}_{CuL} - pK^{H}_{HL}$ for each diastereomeric pair is about one-third of the way between the Gly-X to X-Gly value for both X = Ala and X = Leu.

Upon deprotonation of $Cu(L)^{+}$ and coordination at the amide nitrogen according to eq 9, the corresponding equilibrium acidity constant pK^{H}_{CuL} is more acidic for the pure than for the mixed isomers. The value of pK^{H}_{CuL} for each L,D pair comes close to that of the corresponding Gly-X complex. Thus the high pK^{H}_{CuL} value for Gly-Leu ascribed above to steric inhibition of peptide hydrogen deprotonation also occurs with L-Leu-D-Leu, but not with L-Leu-L-Leu. Evidently hydrophobic interactions between side chains on the same side of the chelate plane in the L,L complex provide a compensating favorable interaction. Such hydrophobic side chain interactions are known to occur also in mixed ligand complexes.[173]

Equilibrium constants have recently been reported for the Ni^{2+}, Cu^{2+}, and Zn^{2+} complexes of a wide range of diastereomeric dipeptide pairs.[174]

C. Side-Chain Dispositions

Tridentate dipeptides chelated to transition-metal ions by amino and deprotonated amide nitrogens and a carboxylate oxygen provide a rigid, planar backbone to which amino acid side chains may be attached. If Pd^{2+} and Ni^{2+} (in the case of tripeptides) are chosen, their diamagnetism allows an estimate of solution conformation of nonchelated side chains by analysis of vicinal proton coupling constants about the α-CH–β-CH_2 bond by nuclear magnetic resonance spectroscopy.[175] Of the three staggered ethanic rotamers about the α,β side chain bond, the rotamer with anti carbonyl and side-chain groups predominates in the free ligands.

For phenylalanine or tyrosine side chains in non-amino-terminal positions of fully formed di- or tripeptide complexes of Pd^{2+} or Ni^{2+}, about two-thirds of the side chains unexpectedly adopt the rotamer with all gauche hydrogens which orients the side chain toward the metal ion.[176,177] The mole fraction of this rotamer, from $^1/_3$ to $^1/_2$, is less in amino acids and peptides with amino terminal aromatic side chains but still greater than in the free ligand. In the L-Phe-L-Phe complex of Pd^{2+}, 61% of the carboxylate terminal and 22% of the amino terminal phenylalanine side chains orient toward the metal ion.[177] The lesser tendency of amino terminal aromatic side chains to orient toward the metal ion agrees with the higher side chain mobility inferred from the low magnitudes of the circular dichroism spectra of the same or similar complexes (section IXB). Only the amino terminal nitrogen is tetrahedral while the trigonal, deprotonated peptide nitrogens provide a stiff backbone.

In the tridentate Gly-Phe and Gly-Tyr complexes of Pd^{2+}, the fourth tetragonal coordination position may be occupied by N-7 of a nucleic purine base. Addition of inosine, 5′-inosine monophosphate, or 5′-guanosine monophosphate does not affect the $^2/_3$ mole fraction of aromatic side chains oriented toward Pd^{2+},[178] nor are the proton chemical shifts of phenolic ring altered. Thus addition of a nucleic base to the fourth coordination position does not affect the aromatic amino acid side chain conformation. On the other hand, the aromatic amino acid side chain induces upfield shifts in the purine bases at H-8 for N-7 binding and at H-2 for N-1 binding.[178] Similar results have been obtained for adenine nucleotides.[179] Thus there is a lack of reciprocity: binding of nucleic base does not affect the conformation or chemical shifts of the aromatic side chain, but nucleic base protons undergo upfield shifts. This mutual result can be explained by the aromatic amino acid side chain residing over the Pd^{2+} and nearly parallel to the chelate plane while the nucleic base binds edge-on perpendicular to the face of the aromatic amino acid.

The rotamer with the side chain oriented toward Cu^{2+} appears in several crystal structure determinations of fully chelated dipeptide and amino acid complexes. There are possible aromatic side chain–Cu^{2+} interactions in a Gly-Trp[93] and L-Tyr complexes,[180] but no interaction in Val-Tyr[95] and Leu-Tyr[94] complexes. In the last complex a water molecule occupies a pyramidal position on the same side of the chelate plane and still does not prevent the tyrosyl side chain from adopting the all-gauche rotamer. A crystal structure also reveals a possible Pd^{2+}–aromatic side chain interaction in a bis(L-tyrosine) complex.[181]

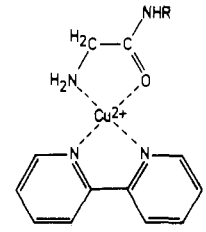
The favoring of the rotamer which orients the side chain toward the metal ion occurs not only with aromatic side chains but also in complexes with the isopropyl and *sec*-butyl side chains of valine and isoleucine.[177] In the tridentate L-Val-L-Val complex of Pd^{2+} *both* isopropyl side chains adopt rotamers that orient methyl groups toward the metal ion. This result supports further the proposed hydrophobic interaction between the isobutyl side chains of L-Leu-L-Leu mentioned in section VB.

There is a demonstrated strong tendency for aromatic and aliphatic side chains in fully chelated amino acid, dipeptide, and tripeptide complexes to take up the staggered ethanic rotamer, which orients the side chain toward the transition metal ions Ni^{2+}, Pd^{2+}, and Cu^{2+}. Crystal structures show this conformation even in the presence of a pyramidal water molecule on the same side of the Cu^{2+} chelate plane. This favored rotamer which places the α-CH–β-CH_2 hydrogens in an all gauche position also occurs in both solution and crystals of cysteine, cystine, and derivatives.[182]

VI. Ternary Complexes Containing Peptides

A. Glycyl Peptides

In sections IVA and VA we have already seen that in the initially formed complex $Cu(L)^+$ between Cu^{2+} and glycylglycinate (or related ligands) the coordination occurs via the terminal amino group and the oxygen of the neighboring amide group (structure **22**). In agreement with this concept, stabilities of ternary Cu^{2+} complexes with 2,2′-bipyridyl are largely independent of the type of the second and following amino acid residues. For the Cu^{2+} complexes with glycinamide, glycylglycinate, glycylglycylamide, triglycine, or tetraglycine the stabilities are similar: $\log K^{Cu(Bpy)}{}_{Cu(Bpy)L} = 5.0 \pm 0.1$.[34,80,81] This result agrees with the suggested structure for all these mixed ligand complexes (**27**).[80,81,183]



27

Also agreeing with structure **27** is the position of equilibrium 16 for all these mixed ligand systems. The

$$Cu(bpy) + CuL \rightleftharpoons Cu(bpy)L + Cu \qquad (16)$$

logarithm of the corresponding equilibrium constant follows from eq 17:[34,36]

$$\Delta \log K_{Cu} = \log K^{Cu(bpy)}{}_{Cu(bpy)L} - \log K^{Cu}{}_{CuL} \qquad (17)$$

The value expected for $\Delta \log K_{Cu}$ on a statistical basis[34] for the present systems is about −0.9, while the experimentally determined one is close to −0.3 throughout.[80,81] This difference means that these mixed-ligand complexes are more stable than expected; this result agrees with the observations generally made for ternary com-

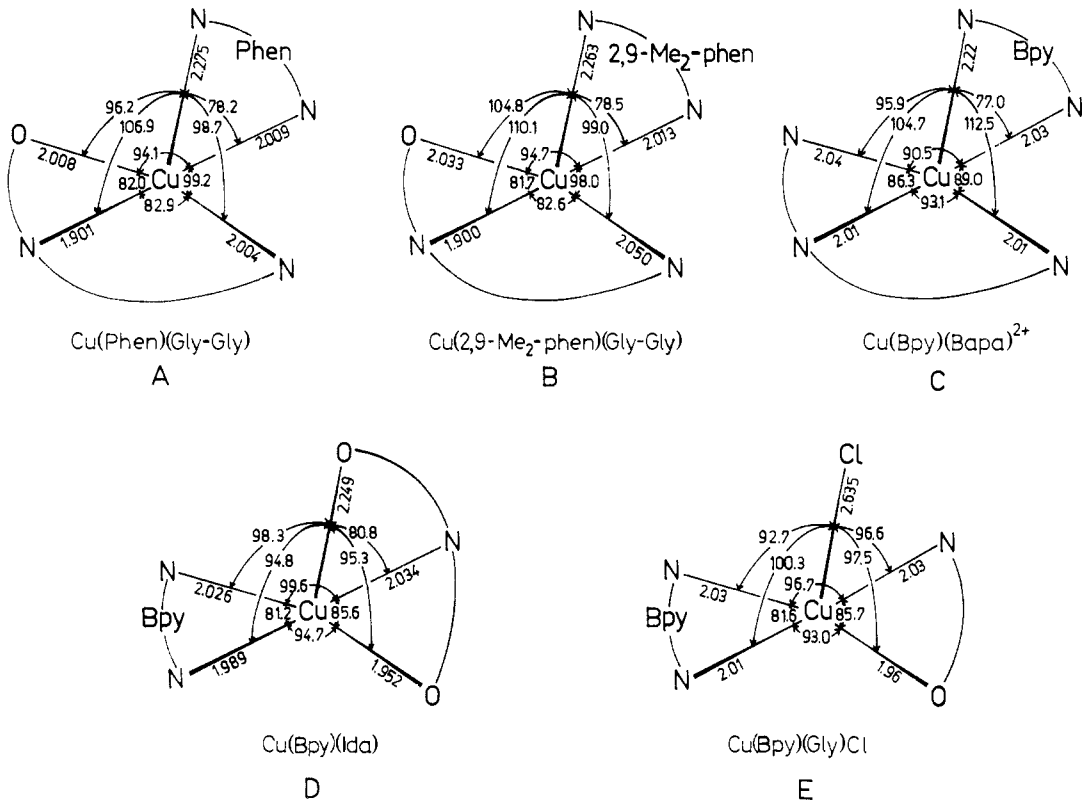
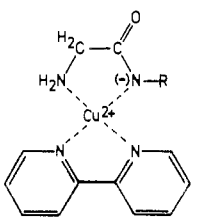


Figure 5. Comparison of the coordination geometries of 5-fold coordinated Cu^{2+} in (1,10-phenanthroline)(glycylglycinato)copper (**A**),[186] (2,9-dimethyl-1,10-phenanthroline)(glycylglycinato)copper (**B**),[187] (2,2′-bipyridyl)[bis(3-aminopropyl)amine]copper(2+) (**C**),[188] (2,2′-bipyridyl)(iminodiacetato)copper (**D**),[189] and (2,2′-bipyridyl)(glycinato)(chloro)copper (**E**).[190] The bond distances are given in Å and the angles in degrees.

plexes consisting of Cu^{2+}, a heteroaromatic nitrogen base, and a second N,O donor ligand,[33-36] as well as with the results (cf. ref 80) obtained for ternary peptide systems with glycinate[184] or ethylenediamine;[185] these latter complexes are less stable.

However, as pointed out already in section IVA, the stability of the binary Cu(L–H) complexes depends strongly on the ligating properties of the additionally involved groups (see structures **8**, **23**, and **24**): the values of pK^{H}_{CuL} differ by 3 log units (Table I). For the mixed-ligand complexes of 2,2′-bipyridyl and the mentioned glycyl derivatives the situation is very different; the values of $pK^{H}_{Cu(Bpy)L}$ are quite similar: L = glycinamide,[31] 7.71 ± 0.06; glycylglycinate,[81] 7.77 ± 0.04; glycylglycylamide,[80] ~7.4; triglycinate,[80] 8.17 ± 0.13; tetraglycinate,[80] ~8.5.

This near consistency is explained by structure **28**.



28

In this structure 2,2′-bipyridyl, the amino group, and the ionized amide nitrogen coordinate to the square-planar positions of Cu^{2+}. As none of the other potential binding sites take part in the coordination, structure **28** can explain the similarity of the values measured for $pK^{H}_{Cu(bpy)L}$.[80]

However, structure **28** contrasts with the crystal structure analysis of the ternary (1,10-phenanthroline)(glycylglycinato)copper(II) complex.[186] In the solid state, the glycylglycine dianion coordinates with all three binding sites, including the carboxylate group, to the square plane around Cu^{2+}. While one of the phenanthroline nitrogens completes the square, the other occupies a tilted apical position, giving a distorted square-pyramidal geometry about Cu^{2+} (Figure 5A). Both the absorption spectrum[186] and calorimetric results[183] support occurrence of the 5-coordinate structure in solution. The answer to this apparent contradiction between structure **28** and structure A of Figure 5 probably lies in an intramolecular equilibrium between different isomers in solution.

An isomer similar to the structure of Figure 5A with a coordinated carboxylate group occurring to 20% in equilibrium would alter the $pK^{H}_{Cu(bpy)L}$ value of Cu(bpy)(glycylglycinate)$^{+}$, compared with the one of Cu(bpy)(glycylamide)$^{2+}$, only by about 0.1 log unit (cf. eq 7), a figure within the experimental error of the measurements; even 30% of such a "closed" isomer would correspond only to about 0.15 log unit. On the other hand, one has also to consider that if an isomer of Cu(bpy)(L–H) in these peptide systems with three binding positions in the square plane of Cu^{2+} would be regularly available for the coordination of the peptide, one would expect that, for example, with triglycine the second amide moiety would also be ionized at higher pH values by forming Cu(bpy)(L–2H)$^{-}$. Such a deprotonation reaction does not occur.[80] An increase in pH with the deprotonation of additional amide groups leads to a displacement of 2,2′-bipyridyl. Hence, one has to conclude that the question for the dominating isomer in solution, i.e., structure **28** or Figure 5A, is not yet solved.

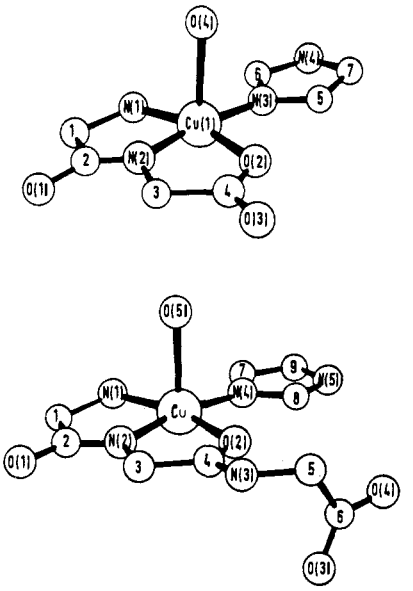


Figure 6. Structures of the (imidazole)(glycylglycinate-H)copper (top) and (imidazole)(glycylglycylglycinate-H)copper (bottom) complexes. The apical position is in both complexes occupied by a water molecule with a Cu–O bond length of 2.4 Å; the average of all the other bond lengths to Cu is 1.98 Å. Reproduced with permission from ref 102. Copyright 1969, Royal Society of Chemistry.

That the coordination sphere of Cu^{2+} is quite adaptable to "outside" restrictions is evident from structures B–E of Figure 5. Similar to the structure of the Cu(Phen)(glycylglycinato) complex (Figure 5A)[186] there is a nitrogen of the heteroaromatic nitrogen base coordinated to the apical position of Cu^{2+} in the ternary complexes (2,9-dimethyl-1,10-phenanthroline)(glycylglycinato)copper (Figure 5B),[187] (2,2′-bipyridyl)[bis(3-aminopropyl)amine]copper(2+) (Figure 5C),[188] (2,2′-bipyridyl)[bis(2-aminoethyl)amine]copper(2+), and (1,10-phenanthroline)[bis(2-aminoethyl)amine]copper-(2+),[191] while the structures of (2,2′-bipyridyl)(iminodiacetate)copper (Figure 5D)[189] and of chloro(2,2′-bipyridyl)(glycinato)copper (Figure 5E)[190] are more similar to structure **28**.

The structures in Figure 5 are consistent with the expected decreasing tendencies to occupy the apical position: $Cl^- > COO^-$ (nonpeptide) > N (aromatic) > N (amino). The dipeptide COO^- is restricted by planarity of the amide bond from attaining an apical position in structures A and B. In structure C the amino nitrogen of bis(3-aminopropyl)amine prefers equatorial positions more than does an aromatic bipyridyl nitrogen. In structure D the tetrahedral amino nitrogen permits rotation of a carboxylate oxygen of iminodiacetate into an apical position.

The structures of the complexes (imidazole)(glycylglycinate-H)copper and (imidazole)(glycylglycylglycinate-H)copper appear in Figure 6. The authors[102] conclude that one of the features of these two complexes is that "the tendency of the peptide and imidazole ligands ... to be coplanar suggests that an imidazole ring prefers to lie close to the plane of the four strongest Cu–ligand bonds unless it is rotated out of the plane by steric hindrance or by hydrogen-bond formation". The Cu–N(imidazole) bond lengths of 1.95 Å are significantly shorter than those of 2.00 Å found in most Cu^{2+}–imidazole complexes.[52,102] This bond shortening agrees with the expected π back-bonding from Cu^{2+} to the empty π orbitals of heteroaromatic nitrogen bases, which was proposed to be important for the stability of such ternary complexes.[33–36,192]

B. Side-Chain-Containing Dipeptides

The stability in solution of the ternary $Cu(bpy)L^+$ complexes formed by dipeptides parallels the observations discussed in section VA with the corresponding binary $Cu(L)^+$ complexes.[81,154] For the Gly-X dipeptides (listed in Table III) the stability constant for amino nitrogen and amide oxygen chelation is 0.01 to 0.18 log unit weaker in the ternary 2,2′-bipyridyl than in the binary complexes. For the X-Gly dipeptides the difference is greater: −0.6 to −0.9 log unit. The Gly-X dipeptides thus show about a 5 times greater tendency to form mixed complexes with bipyridyl.

The nearly constant differences among each class of dipeptides means that the same conclusions reached for the binary complexes apply also to the ternary complexes. The Gly-X systems show little steric or apical interaction effects while in the X-Gly systems there is a steric effect reducing the stability of the Leu-Gly and Ile-Gly complexes and apical interactions increasing the stability of Cys(Me)-Gly, Ser-Gly, and Thr-Gly systems.[154]

In discussing the stability of the mixed ligand species Cu(bpy)(L–H) one first notes that the values of $pK^H_{Cu(bpy)L}$ for glycinamide and glycylglycinate are identical within experimental error.[80,81] This means, contrary to the observations in the binary system (section VA), that the diglycine carboxylate group lacks influence on the stability of Cu(bpy)(L–H). By using the Gly-Gly system with $pK^H_{Cu(bpy)L} = 7.77$ for further comparisons, one arrives at the conclusions[81] already given for the binary systems: an alkyl substituent at the X residue of a X-Gly dipeptide facilitates the ionization of the amide proton, while the same substituent at the X residue in Gly-X decreases the tendency for ionization.

The deprotonation of the amide proton occurs in the Gly-X dipeptides, where X = Ser, Thr, Met, or *S*-methylcysteine, at somewhat lower pH ($pK^H_{Cu(bpy)L}$ = 7.6–8.05)[154] than with glycyl-L-leucinate ($pK^H_{Cu(bpy)L}$ = 8.58);[81] as a result of this, the acidity constants of these dipeptide complexes are rather close to that of Cu(bpy)(Gly-Gly)$^+$. The ternary complexes with the corresponding X-Gly dipeptides are more acidic ($pK^H_{Cu(bpy)L}$ = 5.35–5.81)[154] than those with glycylglycinate or even with L-leucylglycinate (pK^H_{HL} = 6.33),[81] indicating an increased stability of Cu(bpy)(L–H).

Several additional mixed ligand complexes containing peptides have been described;[193,194] among them are those with Ni^{2+} (cf. ref 195, 196), Cu^{2+} (cf. ref 183, 196–203), Zn^{2+} (cf. ref 201), Cd^{2+} (cf. ref 201), and Pd^{2+} (cf. ref 121, 153), and glycylglycine,[195,197,199,202] DL-alanyl-DL-alanine,[199] glycyl-L-leucine,[198,202] glycylsarcosine,[203] glycyl-L-asparagine,[183] glycyl-L-aspartic acid,[183] glycyl-L-glutamic acid,[183] carnosine,[201] diglycyl-L-histidine.[196,200]

VII. Rates and Mechanism

A. Metal Ion Promoted and Inhibited Hydrolysis of Amide Bonds

When coordinated to an amide oxygen, metal ions

promote the hydrolysis of an amide bond. When substituting for a hydrogen at an amide nitrogen, metal ions inhibit the hydrolysis of amide bonds. Indeed, the hydrolysis of an amide bond metalated at a deprotonated amide nitrogen proceeds so slowly that its rate has never been measured.

Several studies bear out these generalizations. At 85 °C the hydrolysis of glycylglycine in the presence of Cu^{2+} passes through a maximum at pH 4.2–4.4.[204,205] The maximum is explained by the rise and fall of the concentration of the amino–carbonyl oxygen chelated complex shown in structure **22**. On the basic side of the maximum, the fully tridentate Gly-Gly chelate with a deprotonated peptide nitrogen donor (**23**) does not undergo hydrolysis. No maximum was observed with Zn^{2+}, only a rise in rate with increasing pH,[205] which agrees with the conclusion that Zn^{2+} cannot deprotonate the peptide nitrogen in Gly-Gly (section IVE). Cu^{2+} inhibits hydrolysis of excess Gly-Gly in strong base; the still observed hydrolysis was ascribed to excess ligand.[206] Also in strong base both Cu^{2+} and Ni^{2+} chelate with hexaglycine and pentaglycine and yield as hydrolysis products glycine and tetraglycine.[207] Both tetragonal metal ions form a quadridentate chelate with the amino group and three deprotonated peptide nitrogens, protecting the amino terminal tetraglycine portion from hydrolysis, which occurs at the dangling uncoordinated peptide groups.

The deprotonated amide nitrogen rather than the additional chelate ring in tridentate Gly-Gly causes hydrolysis inhibition. Cu^{2+} promotes hydrolysis of glycinamide at low pH, where it forms a bidentate chelate through the amino nitrogen and amide carbonyl oxygen (**7**), and inhibits hydrolysis at high pH, where it is bidentate through the amino and deprotonated amide nitrogens (**8**).[38] Cu^{2+} also inhibits hydrolysis of picolinamide (**10**) at high pH.[38]

Basic hydrolysis of amides proceeds by nucleophilic attack of H_2O or OH^- on the amide group carbon atom. Coordinating a metal ion to the amide oxygen increases the positive charge density on the amide carbon and increases its susceptibility to nucleophilic attack in neutral solutions. The zinc metalloenzyme carboxypeptidase may catalyze hydrolysis of peptide bonds in part by this mechanism.[23,208,209] Since metal ions are much less polarizing than protons,[210] substituting a metal ion for an amide hydrogen reduces the susceptibility of the amide carbon to nucleophilic attack to the vanishing point.

The conclusions stated in the first paragraph of this section concerning metal ion promotion and inhibition of amide bond hydrolysis also apply to complexes of nonlabile Co^{3+}. Its inertness enables experiments that reveal details of reactions occurring with coordinated ligands.

cis-β-[(Triethylenetetramine)Co^{III}(OH)(H_2O)]$^{2+}$ chelates to the terminal amino nitrogen and the first carbonyl oxygen of peptides, and amide bond hydrolysis leaves the amino terminal amino acid chelated in [(trien)Co^{III}(amino acid)]$^{2+}$.[211] The reaction proceeds by nucleophilic attack of hydroxide ion at the amide group carbon bound to the chelated oxygen.[212] Intramolecular attack of Co^{3+}-bound OH^- or water on a dangling peptide cis coordinated only at the amino group also yields the chelated amino terminal amino acid.[213]

Peptide bonds may be formed in nonaqueous solvents by subjecting a glycine ester chelated to Co^{3+} by the amino and carbonyl oxygen atoms to nucleophilic attack at the ester carbon atom by the unbound amino group of a glycyl peptide ester.[214] The peptide ester condenses with the chelated glycine ester, the carbonyl oxygen of which remains coordinated and becomes part of the amide bond.

The entire subject of metal ion promoted hydrolysis of amino acid esters and peptides has been reviewed.[215]

B. Metal Ion Deprotonated Amide Bond Formation

Reaction rates and mechanisms for destruction of metal ion–peptide complexes have been investigated by Margerum and co-workers.[216] Peptide complexes of Cu^{2+} and Ni^{2+} undergo nucleophilic substitution in an equatorial position by ligands such as triethylenetetramine (ref 117, 152, 185, 217–220). Amine nitrogens on more sterically crowded ligands such as $EDTA^{4-}$ are poor nucleophiles, but exchange of Cu^{2+} from the peptide complex to $EDTA^{4-}$ is effectively catalyzed by polyamines and amino acid anions.[221] An intermediate mixed complex with polyamine or anionic amino acid reduces the denticity of the coordinated peptide and aids nucleophilic attack by $EDTA^{4-}$.

Addition of base forms amide deprotonated polypeptide metal ion complexes, and addition of acid destroys them. General acid catalysis of the breakdown of the complex is usually observed with Cu^{2+} complexes (ref 152, 220, 222, 223), infrequently with Ni^{2+} (ref 86, 149, 151, 224, 225), and never with Pd^{2+}.[150] These results led to the following overall mechanism.[151,216]

$$M[L\text{-}nH] + HX \underset{k_2}{\overset{k_1}{\rightleftharpoons}} M[L\text{-}nH]\cdot H + X \qquad (18)$$

$$M[L\text{-}nH]\cdot H \xrightarrow{k_3} M[L(-n+1)H] \qquad (19)$$

With a low steady-state concentration of the intermediate M[L-*n*H]·H the observed first-order rate constant is

$$k_{obsd} = \frac{k_1 k_3 [HX]}{k_2[X] + k_3} \qquad (20)$$

When the back reaction is slow compared to decomposition of the intermediate, $k_2[X]/k_3 < 1$, one obtains $k_{obsd} = k_1[HX]$, and the first step is rate limiting and describes general acid catalysis (eq 18). For more inert complexes the decomposition step k_3 (eq 19) proceeds slowly compared to the back reaction k_2, the first step is now at equilibrium, and $k_{obsd} = k_1 k_3 [H]/k_2 K_a$ where K_a is the acidity constant of the general acid catalyst. The reaction then appears specific acid catalyzed, and the second or decomposition step is rate limiting. Though it has not been observed, it should be possible, in a suitably chosen system,[226] to effect a change in rate-limiting step from reaction 18 to reaction 19 by increasing the general catalyst concentration so that while initially $k_2[X] < k_3$, eventually $k_2[X] > k_3$ in the denominator of expression 20 for the observed first-order rate constant.

Margerum and co-workers have proposed that reaction 18 is a simple proton transfer and that the intermediate M(L-*n*H)·H is protonated on a peptide oxy-

gen.[151,216] As they point out, this assignment raises two difficulties. The reaction with hydronium ion, H_3O^+, as a general acid catalyst with the Cu^{2+} and Ni^{2+} complexes of amide-deprotonated triglycine proceeds about 10^5 to 10^6 times slower than the expected encounter-controlled limit. Second, since the point for water lies about 4.7 log units above the line of unit slope set by the Brønsted plot of second-order rate constant vs. pK_a of HX,[86,222] it is necessary to introduce a separate nucleophilic pathway for the water reaction.[216] Water is, however, a poor nucleophile, much weaker than hydroxide, for which no nucleophilic pathway is introduced.

Alternatively, we propose a mechanism in which reaction 18 is general acid catalysis concerted with metal–nitrogen bond breaking. With the same seven general acid catalysts from H_3O^+ of $pK_a = -1.7$ to water of $pK_a = 15.7$ for decomposition of the Cu^{2+} complex of triglycine with two ionized amide groups,[216,222] we find that the Brønsted plot gives a straight line of slope = 0.66 ± 0.07. Furthermore, reaction 18 has been studied in the reverse direction for three general base catalysts, water, borate, and hydroxide, from which we find the slope of the Brønsted plot = 0.33 ± 0.01. The sum of the slopes from the two Brønsted plots falls within experimental error of unity, as required if this analysis is to be correct. In this mechanism proton addition to the complex gives an intermediate in which peptide nitrogen–metal ion bond breaking has occurred.

This alternative mechanism is also consistent with results obtained on complexes of N^{α}-(2-pyridylmethyl)glycinamide and similarly constituted ligands.[227] For the Cu^{2+} complex of the named ligand the authors propose two pathways for its formation and decomposition. The alternative interpretation identifies their k_3 pathway as general acid catalysis by water and unites their two pathways into the single pathway of reactions 18 and 19. For three general acids, H_3O^+, *m*-phenylenediamine, and water, the Brønsted coefficient $\alpha = 0.46 \pm 0.01$, while for three general bases, OH^-, borate, and water, the Brønsted coefficient $\beta = 0.52 \pm 0.04$. For the ethylamide derivative of the above ligand with the same general acid–base catalysts the Brønsted coefficients are $\alpha = 0.42 \pm 0.02$ and $\beta = 0.57 \pm 0.01$. For both ligands the sum of the Brønsted coefficients α and β is again within experimental error of unity.

In the alternative mechanism peptide nitrogen–metal ion bond breaking is concerted with protonation, most likely at the peptide oxygen. The reverse or complexation reaction must follow the reverse pathway. For triglycine chelation the k_2 step is rate limiting. In the complexation direction a preequilibrium produces an O-protonated, N-deprotonated peptide bond tautomer followed by rate-limiting general base catalyzed removal of the oxygen-bound hydrogen concerted with coordination of the peptide nitrogen. About 1 in 10^8 molecules possess the requisite O-protonated, N-deprotonated peptide bond tautomer (section IIC). This fraction does not appear inconsistent with observed rates.

VIII. Trivalent Cu^{3+} and Ni^{3+} Peptide Complexes

In comparison to other donor atoms, deprotonated peptide nitrogens aid formation of relatively stable complexes of Cu^{3+} and Ni^{3+}. Complexes with 3+ metal ions may be made by oxidation of the 2+ complexes by oxidizing agents such as $IrCl_6^{2-}$ and by bulk electrolysis.[228,229] The reduction potential for reaction 21 with

$$M^{III}(GGGG\text{-}3H)^- + e^- \rightleftharpoons M^{II}(GGGG\text{-}3H)^{2-} \quad (21)$$

tetraglycine ligand with three deprotonated peptide nitrogens [$(GGGG\text{-}3H)^{4-}$] is, at 25 °C, $E° = 0.63$ V[230] for Cu and $E° = 0.79$ V for Ni.[231] These potentials are accessible in biological systems.

The oxidized, diamagnetic Cu^{3+} complexes react more slowly than their Cu^{2+} counterparts with acid and nucleophiles; they are unstable with respect to internal oxidation–reduction reactions. The tetraglycine complex of Cu^{3+} with three deprotonated peptide nitrogens has a 5.5-h half-life in neutral solutions at 25 °C.[232]

The reduction-potential $E°$ values depend upon coordinating group, peptide ligand, and solvent. The effectiveness of coordinating groups in stabilizing Cu^{3+} decreases in the order peptide $N^- > NH_2 > OH^- > COO^-$ > imidazole. For Cu and a variety of peptide ligands a range of $E°$ from 0.45 to 1.05 V was found, with bulky side chains favoring lower potentials and Cu^{3+}.[230] The d^8 Ni^{2+} and Cu^{3+} complexes are more strongly tetragonal than the oxidized Ni^{3+} or reduced Cu^{2+} complexes. For this reason introduction of a solvent that reduces coordination in apical positions favors Ni^{2+} and Cu^{3+}, increasing the reduction potential in Ni complexes and decreasing it in the Cu system.[233] Thus, a hydrophobic environment in a protein promises to reduce the reduction potential and renders the Cu^{3+} state more accessible than in aqueous solution.

A recent review article elaborates these and other aspects of Cu^{3+} complexes, with emphasis on peptide ligands.[234]

It has been suggested that the copper enzyme galactose oxidase contains Cu^{3+}, which reacts with D-galactose to give the Cu^+ form of the enzyme and an aldehyde of the sugar substrate.[235] Dioxygen then reoxidizes the Cu^+ form of the enzyme to return the Cu^{3+} galactose oxidase and H_2O_2. This hypothesis presents two difficulties based on studies with small peptides. Even with potential two-electron reductants, Cu^{3+} peptides undergo only one-electron transfer to give Cu^{2+} peptides.[234,236] Second, stabilization of the Cu^{3+} state would seem to require some deprotonated peptide nitrogen donor atoms, but these groups are distinctly unfavorable to formation of Cu^+ complexes. Deprotonated peptide nitrogens appear compatible with a Cu^{3+}/Cu^{2+} couple and other donor atoms such as amino nitrogen, oxygen, and sulfur with the Cu^{2+}/Cu^+ couple. Model compound studies suggest that both of these one-electron couples seem likely prospects for adaptation in proteins. If the two-electron Cu^{3+}/Cu^+ couple appears in proteins, it will be interesting to learn the kind, arrangement, and environment of donor atoms that are present. Two successive one-electron steps via a labile Cu^{2+} intermediate may rapidly undergo the rearrangements required to effect a Cu^{3+}/Cu^+ reaction.

IX. Optical Properties

A. Absorption Spectra

The longest wavelength absorption band of the amide group itself occurs near 220 nm and is due to an $n \rightarrow \pi^*$ transition. Two kinds of transitions to longer

wavelength appear upon coordination of transition-metal ions. Peptide complexes of both Cu^{2+} and Ni^{2+} with deprotonated amide nitrogens give rise to intense charge-transfer absorption near 250 nm.[237] Aromatic peptide side chains also absorb in this wavelength region. Absorption bands with maxima at <290 nm are not considered further in this section.

Ligand field (d-d) transitions taking place predominantly on the metal ion appear at longer wavelength. They occur from about 290 to 330 nm for Pd^{2+}, from 410 to 450 nm for tetragonal, diamagnetic, yellow Ni^{2+}, and from 520 to 650 nm for violet to blue Cu^{2+} complexes. These ligand-field bands, in the visible region for Ni^{2+} and Cu^{2+}, have provided the most useful diagnostic information concerning the structures of transition metal ion–peptide complexes. The subject of absorption spectra in transition metal ion complexes of amino acids and peptides is more fully developed elsewhere.[238]

Substitution of equatorial water molecules in aqueous Cu^{2+} by other oxygen or nitrogen donor atoms moves the absorption maximum to shorter wavelengths, with nitrogen having the stronger effect. There are limitations to quantifying the blue shift because three transitions reside under the absorption band and the same donor atom yields differing effects depending upon chelate ring size and substituents on the ligand. Nevertheless useful generalizations are possible. Consideration of a large number of ligands including glycyl peptides led to an equation for estimating the absorption maximum (λ_{max}) of Cu^{2+} complexes.[239] Expressed in nanometers (nm), the equation becomes

$$\lambda_{max} = 10^3/[0.301(C{=}O/H_2O) + 0.342(COO^-) + 0.453(NH_2) + 0.485(N{=})] \quad (22)$$

The terms in parentheses in eq 22 correspond to the number of donor atoms of each kind to total 4: carbonyl oxygen, water, and hydroxide are enough alike to be considered part of one term, carboxylate oxygen, amino nitrogen, and peptide nitrogen. A separate evaluation gave a parameter of 0.43 μm^{-1} (= 4.3 kK) for each imidazole.[239] We have performed a similar linear multiple regression analysis on a more restricted set of chelated ligands (including non-glycyl derivatives) that are major species in solution so that their absorption maxima are directly measurable. Our result is summarized in eq 23.

$$\lambda_{max} = 10^3/[0.294(C{=}O/H_2O) + 0.346(COO^-) + 0.434(Im) + 0.460(NH_2) + 0.494(N{=})] \quad (23)$$

The two sets of parameters agree closely, and the average difference of about 2% provides an estimate of the reliability of the equations in predicting absorption maxima. The 2% difference corresponds to about 12 nm in wavelength.

It may be more convenient to rewrite the equation in terms of the number of each kind of substituent up to 4 that replaces a H_2O, OH^-, or carbonyl oxygen. Using the parameters from eq 23, we obtain the alternative eq 24 for Cu^{2+} complexes. These equations do

$$\lambda_{max} = 10^3/[1.18 + 0.052(COO^-) + 0.140(Im) + 0.166(NH_2) + 0.200(N{=})] \quad (24)$$

not apply if there is chelation in an apical position. The effects of apical chelation are discussed in section X. The units in all three equations (22–24) are nm.

All three equations agree that the order of increasing ligand field strengths as measured by the ability to effect a blue shift is the same as the order of terms in eq 24. This order should be independent of the metal ion. The deprotonated peptide nitrogen not only exhibits a higher field strength than the amino nitrogen but is also more effective at spin pairing. The bis(1,2-diaminoethane) complex of Ni^{2+} is blue-green, high spin, and octahedral while the bis(glycinamide) complex with two deprotonated amide nitrogens is yellow, low spin, and square planar.[84]

Biuret in strong base with Cu^{2+} forms a bis complex with four deprotonated amide nitrogens (section IIID; structure **21**) of a distinctive violet or biuret color with an absorption maximum at 505 nm.[240] Either eq 23 or 24 predicts an absorption maximum of 506 nm. With peptides and proteins the absorption maximum appears at longer wavelengths: about 515 nm. As more deprotonated peptide nitrogens are substituted about Cu^{2+}, the color gradually changes from blue to violet. The gradualness of the change makes it difficult to specify exact requirements for a biuret color, but most eyes probably require at least three deprotonated peptide nitrogens and a fourth nitrogen donor. With a low molar absorptivity, $\epsilon \sim 100\ M^{-1}\ cm^{-1}$, the biuret test for proteins is relatively insensitive. Though there is no exact correspondence, many ligands that yield a biuret color with Cu^{2+} form diamagnetic, yellow complexes with Ni^{2+}.

Associated with the blue shift upon replacement of oxygen by nitrogen donors about Cu^{2+} is an increasing tetragonality: bond distances to axial ligands become longer.[69] Finally, in the fully chelated tetraglycine[100] and pentaglycine[101] complexes with one amino and three deprotonated peptide nitrogens, and in the bis(biuret) complex with four deprotonated peptide nitrogens,[75] tetragonality becomes complete: there are no axial donors according to crystal structure determinations. This strong tetragonality probably persists in solution. The variable degree of bonding of axial water ligands is automatically included in the aqueous absorption maximum analysis leading to eq 22–24.

B. Circular Dichroism Spectra

Circular dichroism (CD) results from unequal absorption of left and right circularly polarized components of plane-polarized light. No differential absorption appears in optically inactive substances. Even in a strongly optically active material the absorption difference between the two components reaches only about 1% of the total absorption. In this review we use the consistent units, both in $M^{-1}\ cm^{-1}$, of molar absorptivity (ϵ) for absorption and the differential molar absorptivity ($\Delta\epsilon$) between left and right circularly polarized light for CD. The general shape of a CD band due to a single transition resembles that of an absorption band and may possess a negative sign in addition.

With optically active compounds CD spectra often form a valuable adjunct to absorption spectra. Cu^{2+} complexes of peptides (but not amino acids) display a diagnostic CD maximum from 300 to 315 nm, where there is no evident absorption maximum.[237] No ligand-field bands remain to be assigned.[241] The CD band may be due to a magnetic dipole allowed and electric dipole forbidden charge-transfer transition involving a

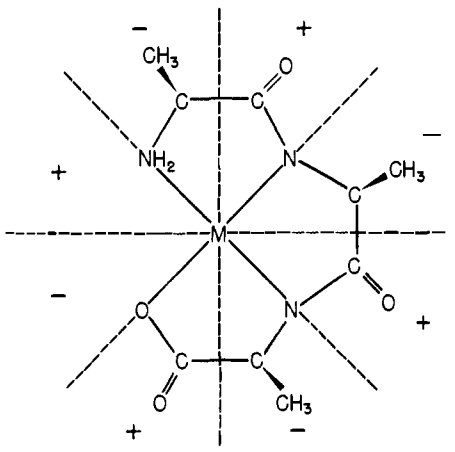


Figure 7. Tetragonal transition metal ion complex of L-Ala-L-Ala-L-Ala with two deprotonated amide nitrogens. The entire complex is in the plane of the paper except for the methyl side chains above the paper plane. Superimposed on the structure are signs for eight hexadecants above the paper plane. Below the paper plane are eight more hexadecants of opposite sign. Reproduced from ref 243.

peptide group and Cu^{2+}. Both Cu^{2+} and Ni^{2+} complexes of peptides exhibit complex CD due to charge-transfer transitions further into the ultraviolet near 250 nm.[237] All subsequent discussion in this section refers to CD through the ligand field (d–d) bands of tetragonal transition metal ion complexes.

Some ligand-field transitions in transition metal ion complexes present favorable cases for observation of CD because they are magnetic dipole allowed and electric dipole forbidden. Thus the ratio of the absorption difference between left and right circularly polarized light to the total intensity may be as large as 1% in an optically active complex. All the ligand-field transitions we shall discuss are electric dipole forbidden. Two of the three transitions that occur under the ligand field absorption bands of Ni^{2+}, Pd^{2+}, and Cu^{2+} complexes are magnetic dipole allowed.[238] We need not distinguish these transitions here, as it is the total net CD intensity under the entire d–d manifold that provides the crucial quantity.[242]

When chelated to a tetragonal transition-metal ion, a di- or tripeptide with deprotonated amide nitrogens provides a rigid framework, as illustrated in Figure 7.[243] The increase in double-bond character in the C–N bond on substitution of a metal ion for the proton reinforces the planarity of the peptide linkage. All non-hydrogen atoms in the peptide backbone and all three chelate rings in Figure 7 are coplanar in the plane of the paper. Amino acid side chains lie above or below this plane depending on whether their configuration is L or D. The known configuration and disposition of amino side chains in the complex of Figure 7 permit tests of sector rules for the vicinal effect of substituents on optical activity. Since the chelate rings of Figure 7 are nearly planar even when there are side chains, the conformational effect due to chelate ring puckering makes a negligible contribution to optical activity.[238,242]

1. Some Results of Amino Acid and Simple Peptide Complexes

CD signs and intensities for ligand field transitions for fully chelated L-alanine tripeptide complexes of Cu^{2+}, Ni^{2+}, and Pd^{2+} appear in Table IV.[120,244,245] The

TABLE IV. Circular Dichroism Intensities in the d-d Region of Tetragonal Complexes of Tripeptides Composed of L-Alanyl Residues[a]

		Cu^{2+}	Ni^{2+}	Pd^{2+}
Ala-Gly-Gly		−0.17	−0.11	−0.10
Gly-Ala-Gly		−0.69	−1.12	−1.50
Gly-Gly-Ala		−0.42	−0.85	−1.00
	sum	−1.28	−2.08	−2.60
Ala-Ala-Ala		−1.19	−2.10	−2.50

[a] Reprinted with permission from ref 238. Copyright 1974, Marcel Dekker, Inc.

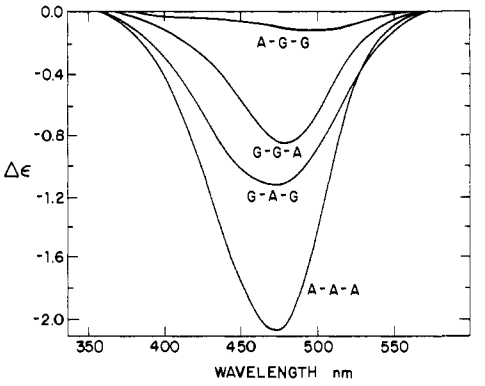


Figure 8. Circular dichroism intensity reported as the difference in molar absorptivity (M^{-1} cm^{-1}) between left and right circularly polarized light vs. wavelength for four Ni^{2+} complexes composed of tripeptides with L-alanine residues.

CD extrema occur at about 560, 480, and 335 nm, respectively, for the three tetragonal metal ion complexes. Figure 8 shows the CD spectra for the L-alanine tripeptide set with Ni^{2+}. The results in Table IV and Figure 8 exhibit several noteworthy features. The signs are all negative for tripeptides containing L-alanine residues. For each metal ion the CD intensity sums for the three tripeptides containing a single L-alanyl residue equal the intensity for the tri-L-alanine complex. Thus there is both sign identity and intensity additivity in the CD of these transition metal ion complexes.[120,243-245] The conclusion does not depend on the methyl side chain: similar results have been obtained for other side chains in tripeptide complexes.[120,244,245]

Representative CD intensities from 590 to 670 nm in 2:1 amino acid and 1:1 dipeptide Cu^{2+} complexes are listed in Table V.[244] Both kinds of complexes are fully chelated; the dipeptide complexes are tridentate with one amino and one deprotonated amide nitrogen and one carboxylate oxygen donor atom. Though most of the signs in Table V are negative, aromatic side chains in amino terminal positions of a dipeptide Cu^{2+} complex give rise to positive CD.

CD intensities for copper complexes of dipeptides containing side chains in both residues are presented in Table VI. Also presented in Table VI are the values calculated by adding the observed $\Delta\epsilon$ values for glycyl-containing dipeptide complexes. For example, the calculated value for the third entry in Table VI is obtained by adding the values for L-alanylglycine and glycyl-L-leucine from Table V. The calculated value for L-Ala-D-Ala in Table VI is obtained by subtracting the value of glycyl-L-alanine from that for L-alanylglycine (both values from Table V). The agreement between the observed and calculated values in Table VI extends to cases where positive values for the X-Gly contribu-

TABLE V. Circular Dichroism Intensitles in Cu^{2+} Complexes of L-Amino Acids (2:1) and Glycyl Dipeptides (1:1)[a]

L-amino acid, X	$\Delta\epsilon$, M^{-1} cm^{-1}		
	2X	Gly-X	X-Gly
alanine	−0.09	−0.35	−0.11
serine	−0.19	−0.31	
asparagine	−0.04	−0.58	
leucine	−0.15	−0.60	−0.08
glutamic acid	−0.16	−0.61	
arginine	−0.14		−0.05
threonine	−0.26	−0.65	−0.07
valine	−0.35	−0.64	−0.09
phenylalanine	−0.44	−0.73	+0.13
tyrosine	−0.45	−0.76	+0.16

[a] Reprinted from ref 244.

TABLE VI. Circular Dichroism Intensities of Cu^{2+} Complexes of Dipeptides Composed of L-Amino Acid Residues[a]

dipeptide	$\Delta\epsilon$, M^{-1} cm^{-1}	
	obsd	calcd[b]
Ala-Ala	−0.47	−0.46
L-Ala-D-Ala	+0.32	+0.24
Ala-Leu	−0.66	−0.71
Leu-Ala	−0.45	−0.43
Leu-Leu	−0.70	−0.68
Arg-Glu	−0.66	−0.66
Val-Phe	−0.76	−0.82
Phe-Val	−0.51	−0.51
Leu-Tyr	−0.88	−0.84
Tyr-Leu	−0.41	−0.44

[a] Reprinted from ref 244. [b] From Table V.

tion are included in the calculation for D-amino acid residues or aromatic side chains. The close agreement demonstrates that the CD intensity of 1:1 Cu^{2+}–dipeptide complexes is an additive function of independent contributions from amino and carboxyl terminal amino acid residues.[244] CD intensity additivity also occurs in Pd^{2+} dipeptide complexes.[120]

2. The Hexadecant Rule and Further Results

The identical CD sign for di- and tripeptide complexes cannot be accounted for by an octant rule for optical activity. An octant or 8-sector rule has been successful in relating configuration to CD sign for the $n \rightarrow \pi^*$ transition in ketones.[246] Octants will not fit on the complex of Figure 7 so that all three amino acid side chains fall into sectors of assigned negative sign. If, however, the octants are doubled into 16 sectors as indicated in Figure 7, all three methyl side chains fall into regions of negative sign.[243] Hexadecants are constructed by dividing the tetragonal plane containing the metal ion perpendicularly into eight wedge-shaped sectors centered on the metal ion. There are 8 sectors of alternating optical activity above the plane of the chelate ring and 8 below, making a total of 16 sectors. All adjacent sectors are assigned opposite signs so that the sign changes on passing perpendicularly through the chelate plane.

The hexadecant rule was developed to account for sign identity and intensity additivity in amino acid and peptide complexes of tetragonal transition metal ion complexes.[243] It represents the pseudoscalar function for the D_{4h} point group which gives the regional rule in the one-electron static dissymmetric perturbation model of optical activity.[247] The microsymmetry of the donor atoms about the tetragonal transition metal ion in Figure 7 approximates D_{4h} symmetry. Tripeptide amides with a third deprotonated amide nitrogen give CD intensities similar to those of the corresponding tripeptides.[244,245] Additional support for the hexadecant rule comes from more recent theoretical calculations along with indications of complications in defining nodal surfaces.[242]

In tripeptides the CD intensity is smallest when the side chain occurs in the amino terminal residue and greatest when it occurs in the middle residue (Table IV). Similarly in dipeptides the smallest intensity occurs for a side chain in the amino terminal residue (Table V). Analysis of complexes with CD intensity additivity suggests that the trend may be accounted for if the ability for the metal ion to sense asymmetry on a nearby side chain is transmitted in decreasing order of effectiveness by peptide nitrogen > peptide C(O)N nitrogen > carboxylate oxygen > amino nitrogen, with the last contribution of positive sign for L side chains. Only the amino nitrogen would not have significant π interactions. Asymmetry with Gly-Ala-Gly is transmitted through the first two donor atoms on the list and is therefore greater than when the methyl side chain is placed in either of the terminal residues.

When located in an amino terminal position of a peptide, aromatic and other bulky side chains require additional consideration. Positive instead of negative CD appears for the L-Phe-Gly and L-Tyr-Gly complexes of Cu^{2+} in Table V. CD intensity additivity, demonstrated for Cu^{2+} complexes of L-alanine tripeptides in Table IV, is also obeyed by the similar set of leucine tripeptides but not for the phenylalanine set.[244] In contrast to other members of the set, the L-Phe-Gly-Gly complex of Cu^{2+} yields a positive $\Delta\epsilon = +0.9$ M^{-1} cm^{-1}. Even with the amino acids phenylalanine and tyrosine, the CD of ternary Cu^{2+} complexes with glycine is 30 to 80% greater than half the value of the 2:1 complex.[241] Complexes with aliphatic side chains fail to produce similar augmentations. A partial explanation for these effects may be found in the side chain dispositions reviewed in section VC. An amino terminal phenylalanine side chain has less tendency to adopt the rotamer that orients the side chain toward the metal ion.[177] In crystal structures of bis(phenylalanine)[248] and bis(tyrosine)[180] Cu^{2+} complexes, one aromatic ring in each complex lies below the chelate plane into a region of opposite hexadecant sign. On the other hand, crystal structures also show an aromatic side chain lying over the Cu^{2+} in the bis(tyrosine) complex[180] and in a glycyltryptophan complex.[93] In these cases a probable direct interaction between the side chain and the metal ion voids applicability of the hexadecant rule.

There are several other situations where peptide complexes composed of L-amino acid residues yield a net positive CD. Net positive CD may be generated in the Cu^{2+} complex of Gly-Gly-L-Ala by raising the pH to 14, where OH^- displaces the chelated carboxylate group. The resulting obligatory rotation about the C–N bond results in a significant change in visible CD pattern.[153] The ability of OH^- to displace the carboxylate group decreases as side chain size increases and a simple rotation becomes sterically improbable. Small upfield chemical shifts upon addition of base to pH 13 in solutions of Pd^{2+} complexes of dipeptides with carboxylate terminal valine and leucine residues have been

attributed to OH^- displacement of chelated carboxylate.[249] The large side chains should inhibit displacement of the carboxylate at pH 13. Substitution of OH^- in the fourth tetragonal position about Pd^{2+} in the dipeptide complex seems to have been ignored. This substitution occurs near pH 9 in the Gly-Gly complex.[120] A new band splits out in the CD spectrum of the Pd^{2+} complexes of leucyl and valyl dipeptide complexes at pH 13.[249] A splitting out also occurs in dipeptide complexes of Cu^{2+} upon deprotonation of water coordinated in the fourth tetragonal position.[241,244]

Cu^{2+} and Ni^{2+} complexes of histidine peptides exhibit a significant longer wavelength negative CD and a shorter wavelength positive CD.[196,250] This pattern, along with the appropriate wavelength maximum in the absorption spectrum, hints at imidazole side chain involvement in peptide complexes.[238] No sector rule has been developed for these irregularly shaped ligands.

X. Apical Amide Coordination in Cu^{2+} Complexes

As concluded in section IVA, the maximum number of ionized amide groups of peptides coordinating to Cu^{2+} in solutions or in the solid state is three, i.e., in complexes with linear oligopeptides Cu^{2+} coordinates to its four equatorial positions the terminal amino group and three nitrogens of the next neighboring amide groups: e.g., in Cu(tetraglycylglycinate-3H)$^{2-}$ the fourth peptide group is *not* deprotonated; neither this nor the terminal carboxylate group play any part in metal ion binding and no axial interaction is observed in the solid state,[101] leaving Cu^{2+} with coordination number four (see also section IXA). This observation agrees (i) with the progressive reduction in the sum of the bond orders of the two axial metal–ligand bonds as the number of ionized N-peptide donors attached to Cu^{2+} increases[69] and (ii) with the planarity of the coordinated ionized amide group, which prevents the last amide unit of Gly-Gly-Gly-Gly-Gly to reach the apical site of Cu^{2+} (section VIA). Therefore, the probability to observe an ionized amide nitrogen coordinated to an apical position of Cu^{2+} seems greater in mixed ligand complexes because ligands with a somewhat lower ligating affinity for the equatorial positions of Cu^{2+} may be selected and the flexibility between two independently coordinated ligands is greater.

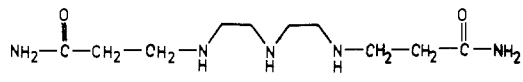
For the Cu^{2+}/diethylenetriamine (Dien)/L-alaninamide (AlaA) system it was suggested that in solution a species Cu(Dien)(AlaA-H)$^+$ with an apically coordinated ionized amide nitrogen is formed.[251] This was concluded from absorption and circular dichroism spectra and a qualitative analysis of potentiometric titrations. However, a careful determination of all the species present in aqueous solutions of the binary parent and the mixed-ligand Cu^{2+} systems, together with the assignment of the corresponding absorption maxima, shows that *no* apically coordinated ionized amide species is formed.[160] In fact, no Cu(Dien)(AlaA-H)$^+$ species could be detected at all; instead at higher pH the "disproportionation" reaction 25 occurs:

$$2\mathrm{Cu(Dien)(AlaA)}^{2+} \rightleftharpoons \mathrm{Cu(Dien)_2}^{2+} + \mathrm{Cu(AlaA\text{-}H)_2} + 2\mathrm{H}^+ \quad (25)$$

Indeed, the reported[251] negative CD may also be attributed to an equatorial coordination of deprotonated L-alaninamide in Cu(AlaA-H)$_2$.

Similarly, Gly-Gly-Gly forms with Cu(Dien)$^{2+}$ also only the ternary Cu(Dien)(GGG)$^+$ complex; again no Cu(Dien)(GGG-H) species could be detected.[185] This contrasts with the results obtained with the tripeptide and the bidentate Cu(En)$^{2+}$: at pH 6.83 the amide-ionized ternary complex Cu(En)(GGG-H) is formed.[185] These observations agree with the conclusions of section IIIE that a bidentate equatorial coordination is crucial for the formation of an ionized *N*-amide-coordinated species.

The diethylenetriamine derivative **29** seems from its



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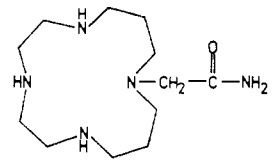
structural design also suitable for an apical interaction because the two terminal amide groups are in positions to form 6-membered rings if the ionized amide nitrogen would coordinate to a metal ion; this together with the flexibility of the tetrahedral amino nitrogens should allow a ligand arrangement involving an apical interaction. However, with Cu^{2+} only the species Cu(L)$^{2+}$ and Cu(L-H)$^+$ could be detected; in the latter complex the ionized amide nitrogen is equatorially coordinated. A species Cu(L-2H), in which an apical interaction appeared as possible, is not observed in the whole pH range up to 12.[252]

Apical chelation in Cu^{2+} complexes produces both a positive CD with L-amino acid derivatives and a red shift of the absorption maximum.[49,239] In neutral solutions L-asparagine chelates to Cu^{2+} equatorially as a substituted glycine with a weak apical interaction of the amide carbonyl oxygen.[49,156,253] Compared to L-alanine complexes there is a small red shift of the visible absorption maximum and a smaller than expected negative CD.[49] Near pH 11 the Cu^{2+} substitutes for an amide hydrogen.[49,253] Upon complete substitution near pH 13 the absorption maximum of the 2:1 complex appears at 580 nm, and there is a net positive CD, which has been compared to that observed in the 2:1 histidinate complex.[49] The results may be explained by four nitrogen donors in the chelate plane and apical chelation of the carboxylate groups. Equation 23 in section IXA predicts 524 nm as the absorption maximum with two amino and two deprotonated amide nitrogen donors. The 56-nm red shift is consistent with apical carboxylate chelation.[239] Amide hydrogen substitution by Cu^{2+} does not occur with glutamine, which requires a 7- rather than a 6-membered chelate ring.[253] With the more strongly binding planar (En)Pd$(H_2O)_2^{2+}$, substitution of an amide hydrogen occurs with both asparagine (pK_a = 6.4) and glutamine (pK_a = 9.0).[254] On the basis of a shift of the absorption maximum from about 630 nm to 570 nm at pH >10 in solutions of Cu^{2+} and glycylasparagine,[183] we conclude that there is a rearrangement from glycylglycinate-like coordination to one amino and two deprotonated amide nitrogen donors in equatorial coordination at high pH. Equations 23 and 24 predict an absorption maximum of 573 nm. The dipeptide carboxylate group becomes unbound.

A series of tetraaza macrocyclic Cu^{2+} complexes with an amide function in a side chain[255–257] have been syn-

thesized; with an *N*-[(aminocarbonyl)methyl] side chain (**30**), amide nitrogen ionization occurs with $pK_a = 9.9$.[255]



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However all these complexes, amide deprotonated or not, do *not* possess the spectral characteristics[256,257] of tetragonal Cu^{2+} complexes with strong equatorial and weak apical coordination sites (section IXA).[239] Unfortunately, structures of these complexes are not yet known.

To summarize, by taking everything together we conclude that there is no indication for an apical coordination of an ionized amide nitrogen in any of the studied binary or mixed-ligand complexes. In tetragonal Cu^{2+} complexes ionization of amide groups is restricted to equatorial positions; an ionized amide nitrogen will always coordinate at the stronger Cu^{2+} sites and displace, where necessary, weaker ligating groups to an apical position.

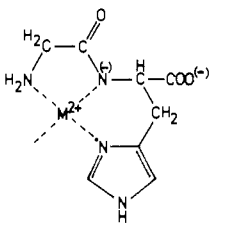
However, the more weakly coordinating neutral amide group is able to coordinate via its oxygen atom at an apical Cu^{2+} site. For $Cu(Dien)(AlaA)^{2+}$ there is evidence that four nitrogens are equatorially coordinated and that the carbonyl oxygen of alaninamide interacts to a significant extent with an apical position.[160] This is hinted by a slightly larger stability of $Cu(Dien)(AlaA)^{2+}$ compared with $Cu(Dien)(NH_3)^{2+}$ and is confirmed by the red shift of about 16 nm of the absorption maximum of $Cu(Dien)(AlaA)^{2+}$ (λ_{max} 592 nm) compared to that of $Cu(Dien)(NH_3)^{2+}$ (576 nm). There is also evidence for apical interactions of other groups (sections VB and VIB)[159] including carboxylate coordination in the Cu^{2+} complexes of 2,4-diaminopropionate[49] and histidinate,[49,258] and in mixed-ligand complexes of dipeptides and amino acids,[183,199] as well as for carboxylate groups of peptide side chains[234] in Cu^{2+}–peptide complexes.

XI. Histidine Peptides

In peptides the imidazole side chain of a histidine residue offers a basic binding site ($pK_a \sim 7$) in a position to form a favorable 6-membered chelate ring with its own deprotonated peptide nitrogen (section IIIC). It has been strongly argued that of the two nitrogens in the imidazole ring, coordination of a single metal ion occurs at the pyridine rather than pyrrole type nitrogen.[52] When not metalated, these two nitrogens undergo rapid tautomeric exchange.[52,156]

A. Glycylhistidine, Diglycylhistidine, and Derivatives

In neutral solutions glycyl-L-histidinate chelates to Cu^{2+} (ref 50, 259), Ni^{2+} (ref 50), and Pd^{2+} (ref 260) as a tridentate ligand with three nitrogen donor atoms: amino, deprotonated peptide, and imidazole (**31**). The carboxylate group will not bind simultaneously to tetragonal Cu^{2+} and Pd^{2+}. Compared to Gly-Gly the imidazole nitrogen steals the third coordination position from the less basic carboxylate group. This structure, originally deduced from potentiometric titrations, has been found in crystal structures of Cu^{2+} complexes of Gly-His,[261] Gly-His-Gly,[262] and Gly-His-Lys.[263] The carboxylate group may be coordinated in the Ni^{2+} complex since the metal ion remains hexacoordinate in neutral solution.[50] With Co^{2+} in oxygen-free solutions, the peptide nitrogen deprotonates with $pK_a = 7.2$.[137,141] A similar pK_a is observed with Zn^{2+}, and the possibility that it represents the peptide proton was mentioned in section IVE.



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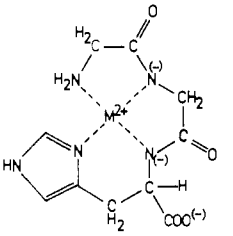
In equimolar solutions of Gly-His and Cu^{2+}, Ni^{2+}, or Pd^{2+} a concentration-dependent deprotonation occurs at about pH 9.6.[260] An accompanying pronounced shift of the d–d absorption band to shorter wavelengths occurs for Cu^{2+} and Pd^{2+}, while the pale green Ni^{2+} complex turns yellow. These changes indicate substitution of oxygen by a nitrogen donor to the metal ions. The deprotonation has been assigned to metal ion substitution of the pyrrole hydrogen on the imidazole ring.[260] The imidazole ring then bridges two metal ions. A detailed pH and spectrophotometric study of the equilibria indicates formation of a polymer, probably a tetramer.[260]

The tripeptide Gly-Gly-L-His has special interest because it serves as a model for the amino terminus of albumins which also bear a histidine residue in the third position (section XV). Gly-Gly-His chelates to Cu^{2+} (ref 196, 259, 264, 265) and Ni^{2+} (ref 196) as a quadridentate ligand with four nitrogen donor atoms: amino, two deprotonated peptide, and imidazole (**32**). The titration curves for the four deprotonations occur over a narrow, highly buffered pH region, and it is difficult to resolve individual peptide deprotonations (see discussion in sections IVA3 and IVB). For the $Cu(HL)^{2+}$ complex successive deprotonation pK_a values of $4.23^{266}/4.65$,[196] $4.87^{266}/5.06$,[196] and $4.60^{266}/4.51$[196] have been reported. The inverted order indicates a high degree of cooperativity in the last two deprotonations which result in $Cu(L\text{-}2H)^-$. Halfway through the titration, half of the ligands are fully complexed and the other half unbound. The amino group at one end of Gly-Gly-His and an imidazole nitrogen at the other end form the termini for three chelate rings only if both intervening peptide nitrogens are deprotonated (**32**). The sum of the two pK_a values ($9.5^{266}/9.6$[196]) is 2 log



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units less than the corresponding sum (11.8) for Gly-Gly-Gly in Table I. Crystal structure determinations of Cu^{2+} complexes of glycylglycylhistidine *N*-methylamide[267] and an oxidative decarboxylated product of Gly-Gly-His[268] show the expected quadridentate chelation. Oxidative decarboxylation has also been suggested to occur in the Ni^{2+} complex of Gly-Gly-His.[269] The combination of an amino terminus with a histidyl residue in the third position presents the strongest peptide binding site for tetragonal metal ions capable of deprotonating peptide nitrogens.

Zn^{2+} probably also promotes peptide nitrogen deprotonation in Gly-Gly-His, but binuclear complexes also form.[265] Structures are less certain than those with tetragonal transition-metal ions.

At high pH with $pK_a = 10.7$ an additional deprotonation occurs in equimolar solutions of Cu^{2+} and Gly-Gly-His.[259] No blue shift accompanies the deprotonation. All equatorial coordination positions about Cu^{2+} are occupied by the quadridentate ligand. The deprotonation is attributed to Cu^{2+} promotion of pyrrole ionization from across the imidazole ring. The pK_a value is more than 1 log unit higher than that mentioned above for Gly-His where an equatorial Cu^{2+} position substitutes for the pyrrole hydrogen.[52]

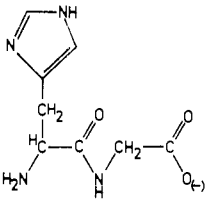
The corresponding amino-acetylated derivatives of Gly-His, Gly-Gly-His, and Gly-Gly-Gly-His have also been investigated for their ability to bind Cu^{2+}.[264] The last ligand serves as a model for an internal histidyl residue in proteins. Peptide deprotonations occur with more difficulty in the acetylated ligands but still take place in neutral solutions.

B. Thyrotropin-Releasing Factor (L-Pyroglutamyl-L-histidyl-L-prolinamide)

Thyrotropin-releasing factor (TRF), L-pyroglutamyl-L-histidyl-L-prolinamide, also contains an acylated histidine residue. Essentially identical solution characteristics have been obtained with L-pyroglutamyl-L-histidine and Cu^{2+} (ref 270) or Ni^{2+} (ref 271), indicating that the L-prolinamide group of TRF is not involved in metal ion binding. Yellow, diamagnetic Ni^{2+} complexes are formed only at high pH (>9), and excess ligand is needed to inhibit precipitation. Only two nitrogen donors were identified in an ESR spectrum of a frozen solution of TRF and Cu^{2+} at pH 9.[270] However, the titration curve for an equimolar solution with Cu^{2+} is highly buffered, with 90% of the addition of 3 equiv of base occurring within 2 pH units.[51] This result suggests cooperative loss of protons from the imidazole and two amide nitrogens. At the end point of the 3 equiv of base at pH 8.5, an absorption maximum appears at 588 nm.[51] This result is more consistent with a maximum at 582 nm predicted from section IXA for one imidazole and two deprotonated amide nitrogen donors than at 659 nm predicted for one imidazole and one amide nitrogen donor. Thus, combined evidence from potentiometric titration and visible absorption spectra indicates that at pH 8.5 TRF chelates Cu^{2+} by three nitrogen donors. The imidazole nitrogen and histidyl and pyroglutamyl deprotonated amide nitrogens chelate to form three fused rings. The visible CD spectrum shows positive peaks. As pointed out in section IXB, this feature is characteristic of chelation to an L-histidyl residue. At still higher pH (>10), a small blue shift in the absorption spectrum results from pyrrole nitrogen deprotonation.

C. Histidylglycine and Histidylhistidine

In neutral solutions L-histidylglycinate (**33**) presents four potential donor atoms, but those from a single ligand cannot coordinate simultaneously to the four



33

coordination positions about a single tetragonal metal ion. We can visualize the dipeptide **33** chelating as a substituted histidine through amino and imidazole nitrogens or as a substituted glycylglycinate through amino and deprotonated amide nitrogens and a carboxylate oxygen. Neither of these chelation modes meets the requirements for induction of a diamagnetic planar Ni^{2+} complex. With Cu^{2+} the former mode is favored at pH 5.[272] Between pH 6 and 7 the peptide hydrogen undergoes substitution, and the latter substituted glycylglycinate mode results. Results of a potentiometric[272] investigation and a combined concentration-dependent potentiometric–spectrophotometric[273] study agree on formation of a dimer. There is near identity of absorption spectra of a solution containing equimolar amounts of His-Gly and Cu^{2+} at pH 8 and one containing the same concentrations of Cu^{2+}, Gly-Gly, and imidazole.[272] Each Cu^{2+} is chelated by His-Gly in a tridentate glycylglycinate mode with a nitrogen from each imidazole coordinated to the available tetragonal position on a second Cu^{2+}.

A match between four potential donors in His-Gly and four tetragonal coordination positions in an equimolar neutral solution with Cu^{2+} is achieved by dimer formation. Equimolar solutions of Gly-His and Cu^{2+} solve the problem of a vacant coordination position at pH 10 by tetramer formation. An evident driving force utilizes available donors and fills vacant coordination positions by polymer formation. The smallest polymer allowed by steric considerations seems favored. The ligand carnosine, to be discussed in section XID, also possesses four potential donors in neutral solutions, and its Cu^{2+} complex takes up a dimer structure similar to that of His-Gly.

Addition of a second imidazole ring to His-Gly gives the dipeptide His-His. Cu^{2+} (cf. ref 50, 274) and Ni^{2+} (cf. ref 50) substitute for the His-His peptide hydrogen in neutral solutions.[50] In the systems with Cu^{2+} and Zn^{2+} binuclear complexes are the major species in the neutral pH range.[274] While amide ionization was confirmed in the presence of Cu^{2+} it was not detected with Zn^{2+}.[274] For the (histidylhistidinate)cobalt(+) complex amide ionization occurs with $pK_a \simeq 7.8$.[137]

D. Carnosine (β-Alanyl-L-histidine), Anserine, and Deprotonation of the Pyrrole Nitrogen

Carnosine (β-alanyl-L-histidine) (**34**) occurs in vertebrate muscle at millimolar concentrations and is of

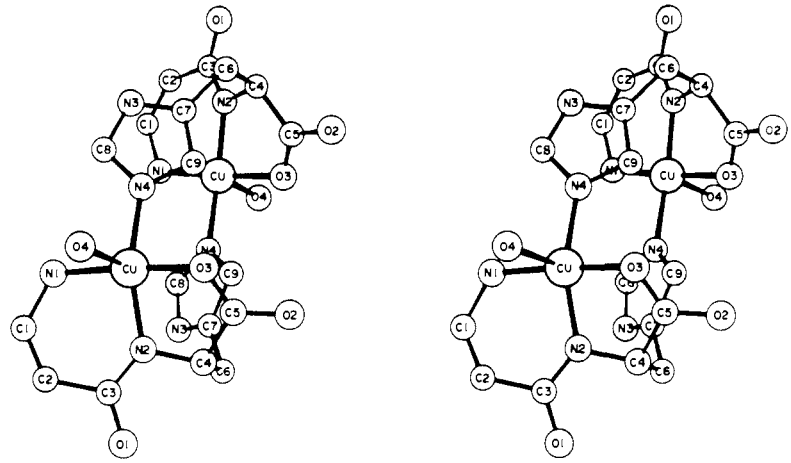
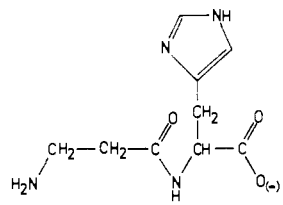


Figure 9. Stereoscopic view of the dimeric carnosine–Cu^{2+} complex. Reproduced with permission from ref 277. Copyright 1967, International Union of Crystallography.

uncertain function. It is a substrate for the metal ion activated hydrolytic enzyme carnosinase.[275] The two successive methylene groups of the β-alanyl residue require a 6-membered ring to chelate the amino and deprotonated peptide nitrogens in **34**. Early problems



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in resolving titration curves of Cu^{2+} and carnosine[276] were solved by the result of a crystal structure determination, which revealed a dimer as shown in Figure 9.[277] Each Cu^{2+} is chelated by amino and deprotonated peptide nitrogens and a carboxylate oxygen, the backbone chelating as would β-alanylglycine. The dimer is joined by an imidazole nitrogen of each ligand coordinating to the fourth tetragonal position on the other Cu^{2+}.

There has been some discussion as to whether the carnosine dimer complex found in the crystal structure determination occurs in solution. Titration curves of equimolar solutions of Cu^{2+} and either carnosine or anserine (β-alanyl-L-1-methylhistidine) are virtually superimposable.[50,276] Peptide nitrogen deprotonation occurs between pH 5 and 6, and an end point is achieved after addition of 2 equiv of base at pH 7. These titration results rule out a glycylhistidinate type structure (**31**) for carnosine because the imidazole 1-nitrogen of anserine is methylated and unavailable for coordination.[52] Since the imidazole 3-nitrogen binds the carnosine dimer, methylation at N-1 has no effect on the dimer. In addition, a second mole of either carnosine or anserine titrates as if unbound and does not affect the circular dichroism.[49] Because all four tetragonal positions about Cu^{2+} are occupied in the dimer, it accounts for the freely titrating additional ligand. The equilibrium constant for dimer formation from monomers in the neutral solutions has been estimated as log K = 3.6 from CD spectra[49] and calculated as log K = 4.16 from potentiometric titration results.[278] These values obtained directly on the Cu^{2+}–carnosine complex agree well with the model system of fully chelated tridentate dipeptide complexes of Cu^{2+} that coordinate free imidazole with log K = 4.0.[279] Thus not only is the presence in solution of the Cu^{2+}–carnosine complex dimer twice verified and reasonable, but also claims for its absence require explanation. We conclude that at concentrations greater than 0.1 mM the carnosine–Cu^{2+} dimer is the predominant species in neutral solutions.

Recent papers from two groups of workers utilizing primarily magnetic resonance spectroscopies[280,281] present little new information on the structure of the Cu^{2+}–carnosine complexes when the previous literature is taken into account. Since the Cu^{2+}–carnosine dimer is not detectable by ESR at room temperature, experiments are conducted with frozen solutions at 77–93 K.[280,281] Because of severe broadening by Cu^{2+}, NMR experiments are conducted at high ratios of carnosine to Cu^{2+}. Under these conditions chelate rings involving a deprotonated amide nitrogen do not close before Cu^{2+} interacts with a carnosine primarily in a unidentate mode at the imidazole ring before passing rapidly onto another molecule (section IVA).[52,108] Broadening of ligand NMR peaks by Cu^{2+} is not dipolar determined and cannot be used to infer structures or to estimate distances to ligand atoms.[108,282]

The pK_a values for pyrrole nitrogen deprotonation in the imidazole ring of the Cu^{2+} complexes support the structures put forth in this section. For Gly-His,[260] Gly-Gly-His (section XIA),[259] His-Gly (section XIC),[264] and carnosine,[283] the imidazole ring pyrrole pK_a values are 9.6, 10.7, 10.6, and 11.2, respectively, all reduced from pK_a = 14.2 in unbound imidazole.[52] As discussed in section XIA the low value for Gly-His results from substitution of the pyrrole hydrogen by Cu^{2+} to form a tetramer. The higher values for the last three ligands are due to Cu^{2+} promoting the deprotonation remotely from across the imidazole ring. Substitution of a metal ion for a pyrrole hydrogen in imidazole is more effective in acidifying the pyrrole hydrogen than is remote, across the imidazole ring, promoted deprotonation.[52] In the complexes of the last three ligands donor atoms occupy all four equatorial positions about Cu^{2+} in neutral solutions. Gly-Gly-His is quadridentate (**32**). His-Gly and carnosine must form dimeric complexes to occupy all four equatorial Cu^{2+} positions. The high pK_a values for the pyrrole deprotonation provides additional proof of the dimer structure. No opportunity exists for Cu^{2+} to substitute for a pyrrole hydrogen in the last three

complexes. Across the imidazole ring promotion of the pyrrole deprotonation is the only possibility.

Why does the additional methylene group cause such drastic differences in the structure of the Cu^{2+} complexes of glycylhistidine (**31**) and carnosine (Figure 9)? In neutral solutions both tridentate ligands chelate by amino and deprotonated peptide nitrogen atoms, but glycylhistidine uses an imidazole nitrogen to form a 6-membered chelate ring and carnosine a carboxylate oxygen to form a 5-membered chelate ring. For carnosine with its β-alanyl residue to adopt the Gly-His complex structure **31** would require formation of two 6-membered chelate rings. The peptide group with its deprotonated nitrogen a part of both rings cannot accommodate the two adjacent 6-membered rings and preserve its planarity.[277] Similarily in solution Cu^{2+} is unable to deprotonate the peptide nitrogen in β-alanyl-β-alanine, a process which would result in two adjacent 6-membered chelate rings.[37]

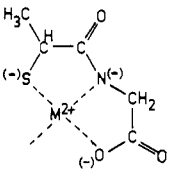
E. Comparison of the Coordinating Properties of the Imidazole Ring and the Amide Group

Since both occur in histidine peptides, it is informative to compare and contrast protonation and metalation of the imidazole ring and the peptide or amide group. We relate the amide nitrogen to the imidazole pyrrole nitrogen and the amide oxygen to the imidazole pyridine nitrogen. Both neutral species have been supposed to be protonated and metalated at the wrong site: the amide nitrogen for amides and the pyrrole nitrogen for imidazole. Protonation and metalation occur at the amide carbonyl oxygen (section IIB) and at the imidazole pyridine nitrogen.[52] Only upon their deprotonation do metal ions coordinate to the amide nitrogen and to the imidazole pyrrole nitrogen. Both neutral molecules deprotonate in the absence of metal ions to give anions until $pK_a > 14$ (section IIA and ref 52). Coordination of Co^{3+} at the amide oxygen and at the imidazole pyridine nitrogen promotes the deprotonation of both a nitrogen-bound amide hydrogen[284] and the imidazole pyrrole hydrogen[285] to $pK_a \sim 10$–11. In contrast, protonation of a neutral imidazole ring occurs with $pK_a \sim 7$ while protonation of amide groups takes place in quite acidic solutions with $pK_a \sim -1$ (section IIB). Thus imidazole serves as a much better ligand for metal ions than do neutral amides. However, loss of the nitrogen proton from a protonated amide to give the rare oxygen-protonated neutral tautomer occurs with $pK_a \sim 7$ (Figure 1) identical with that of imidazole. As a second contrast, with a metal ion at the amide nitrogen, the amide oxygen may protonate with $pK_a \sim 2$ (section IVF) while with a metal ion at an imidazole nitrogen, the loss of a proton across the ring takes place with $pK_a \sim 11$.[52,286] This difference occurs because the electronic distribution within an imidazole ring readjusts so that a single metal ion always coordinates to a pyridine rather than a pyrrole type nitrogen.

XII. Sulfhydryl-Containing Amide Ligands

The ability of the sulfhydryl group to serve as an anchor for metal ion chelation at a deprotonated amide nitrogen has been examined on several compounds. Though some tendency toward amide deprotonation cannot be ruled out, there is no firm evidence for its occurrence at pH < 11 in solutions containing *N*-acetylcysteine and either Ni^{2+} or Pd^{2+}.[287] The main contributor to consumption of additional base in equimolar solutions is probably metal ion hydrolysis. Considerable polynuclear complex formation through sulfhydryl bridging is indicated by an absorption band at 420 nm.

With (α-mercaptopropionyl)glycinate (MPG), Ni^{2+} substitution for the sulfhydryl and amide protons is complete by pH 8 with formation of an assumed mononuclear complex with a terdentate ligand (**35**).[288]



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Later investigation revealed extensive polynuclear complex formation in equimolar solutions as indicated by a strong absorption band at 370 nm due to sulfhydryl bridging.[287] Therefore structure **35** is oversimplified. The 370-nm band disappears at a 3:1 MPG:Ni^{2+} ratio at pH 9, where the complex is diamagnetic. The resulting mononuclear, diamagnetic, planar complex probably contains an additional sulfhydryl group in the fourth position of structure **35**. Pd^{2+} forms similar complexes with a greater tendency to polynuclear complex formation.[287]

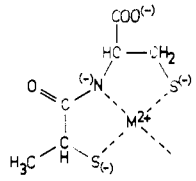
The most outstanding feature of the MPG ligand is its ability to chelate Cu^{2+} as in **35** without reduction and oxidation to Cu^{+} and disulfide.[289] In equimolar solutions amide deprotonation is induced by Cu^{2+} near pH 6.5 with appearance of a green color and absorption maxima at 400 and 605 nm. Features of absorption and ESR spectra are similar to those of intensely blue copper proteins,[289] but the MPG complex is significantly more difficult to reduce to a Cu^{+} state.[290]

It is interesting to compare stability and acidity constants for Cu^{2+} complexes of the extended set of sulfhydryl containing amide ligands, (α-mercaptopropionyl)glycine (MPG), (α-mercaptopropionyl)-β-alanine, (β-mercaptopropionyl)glycine, and (β-mercaptopropionyl)-β-alanine,[291] which form two joined chelate rings of sizes 5/5 (**35**), 5/6, 6/5, and 6/6, respectively, corresponding to the set of dipeptide ligands Gly-Gly, Gly-β-Ala, β-Ala-Gly, and β-Ala-β-Ala of the same respective ring sizes.[37] The sulfhydryl group is slightly more basic than the amino group, and the stability constant for Cu^{2+} in the sulfhydryl set is greater than that for the corresponding member of the dipeptide set. However, the pK_a for amide deprotonation by Cu^{2+} in the sulfhydryl set occurs with $pK_a = 6.2$–7.6 and is about 2.2 log units greater than that for the dipeptide set. The net result is that for the reaction of Cu^{2+} with the ligand as it exists in neutral solutions the dipeptide set actually forms stronger complexes than the sulfhydryl group of amide ligands. On this basis, for both sets of ligands, the stability order for the joined ring systems is 5/5 > 5/6 > 6/5 > 6/6.

For the above set of four sulfhydryl-containing amide ligands stability constants and absorption spectra have

also been reported for the Ni^{2+} complexes.[291] With Ni^{2+}, amide deprotonation occurs with pK_a = 6.9–8.0. For the Ni^{2+} complexes a strong absorption band at 375–412 nm indicates extensive polynuclear complex formation. The possible occurrence of polynuclear complex formation in the Cu^{2+} complexes warrants investigation.

Sulfhydryl-containing amide ligands with an additional sulfhydryl group, (2-mercaptopropionyl)-L-cysteinate, and a histidine residue, *N*-(mercaptoacetyl)-L-histidinate, have been synthesized and their Cu^{2+} and Ni^{2+} complexes investigated.[292] Potentiometric titrations and visible absorption spectra indicate amide deprotonation in neutral to slightly acidic solutions to give chelates with structures **36** and **37**. Once again,



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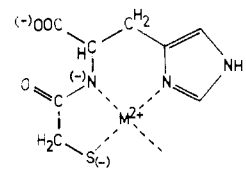

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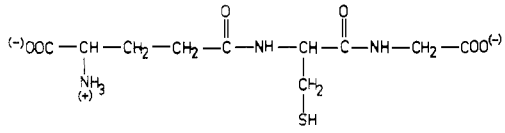
strong absorption in the Ni^{2+} complexes at 410 and 382 nm suggests polynuclear complex formation through sulfhydryl bridges. Two peptide deprotonations occur in the formation of the Cu^{2+} complex of *N*-(mercaptoacetyl)glycyl-L-histidinate to give a complex with three chelate rings.[293] With both a sulfhydryl and an imidazole ring donor, features of these complexes relate to the binding site in intensely blue Cu^{2+} proteins, even though no peptide deprotonations occur in the proteins (section XV). Two peptide deprotonations also occur in the Cu^{2+} complex of *N*-(mercaptoacetyl)-L-tyrosine; the phenolic group is not coordinated.[294]

The tripeptide glutathione (γ-L-glutamyl-L-cysteinylglycine, **38**) contains a central cysteinyl residue and



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serves as a superb model for the sulfhydryl group of a cysteinyl residue in proteins. Both the substituted glycine at the γ-glutamyl locus and the sulfhydryl group provide coordination sites for metal ions.[295,296] When both potentiometric titration and absorption spectra results are considered, chelation of a metal ion between the sulfhydryl group and a deprotonated peptide nitrogen has been found to occur at pH >12 with Ni^{2+} in a red complex and at pH 7–11 with Pd^{2+}.[287] It is suggested that both metal ions form a 6-membered chelate ring with the deprotonated glycyl peptide nitrogen rather than a 5-membered ring with the deprotonated cysteinyl peptide nitrogen. On the basis of small shifts in ^{13}C NMR spectra, it has been proposed that Zn^{2+} chelates to the sulfhydryl group and a deprotonated peptide nitrogen in glutathione from pH 10.5 to 13.[140] This suggestion implies that in glutathione Zn^{2+} substitutes for a peptide proton at a lower pH than Ni^{2+}. It has also been proposed on the basis of small ^{13}C NMR chemical shifts that Hg^{2+} chelates to the sulfhydryl group and a deprotonated peptide nitrogen in glutathione,[297] but this chelation mode is unlikely for Hg^{2+}, and the shifts have been otherwise explained.[296]

With Cu^{2+}, glutathione and other compounds containing sulfhydryl groups undergo an oxidation to give Cu^{+} and a disulfide. Several disulfide-containing amides have been examined for their chelating capabilities. Cu^{2+} substitutes for peptide protons in oxidized glutathione,[298] L-cystinediamide, and L-cystinylbisglycine.[299,300] Though there may be relatively weak interactions between Cu^{2+} and the disulfide bond,[88,170b] the latter does not serve as an effective anchor. In the above disulfides Cu^{2+} chelates to deprotonated peptide nitrogens almost as it would in the absence of the disulfide bond (see also section XIIIA). The separate topic of metal ion interactions with the disulfide bond has been reviewed.[156]

XIII. Polypeptides

Small peptides that are not acylated possess an anchor at the amino terminus for transition metal ion chelation at up to three deprotonated peptide nitrogens on succeeding residues (section IV). Successive pK_a values in Table I indicate that for both Cu^{2+} and Ni^{2+} two peptide nitrogens undergo deprotonation by pH 7 in tetraglycine with the third deprotonation under way from pH 8 to 9. This type of interaction could be general for peptides and proteins.

A. "Heteropeptides"

Similar to tetraglycine, interaction of one cupric ion per molecule of oxytocin liberates 4 equiv of acid to pH 9, finally yielding an absorption maximum at 515 nm.[301] No change in absorption appears throughout the region in which the tyrosine hydroxy group is ionizing. Desaminooxytocin exhibits no complex formation below pH 11, demonstrating the importance of a free amino group for cupric ion interactions in the physiological pH range. Above pH 12 the usual biuret reaction with proteins occurs.

Similar evidence for cupric ion interaction at the N-terminal amino group and subsequent ionization of three neighboring peptide hydrogens is suggested by results of comparative potentiometric and spectrophotometric studies on lysine-vasopressin and (acetyllysine)-vasopressin, where the α-amino group is blocked.[302] Unlike the latter derivative, lysine-vasopressin forms a 1:1 complex with cupric ion in which 3 additional equiv of hydrogen ion are titrated from pH 4 to 8. The higher pK_a values of the phenolic and ε-amino groups are not altered by complex formation. After ionization of three hydrogens, a solution of the 1:1 lysine-vasopressin–cupric ion complex exhibits a maximum at 525 nm.[302] Both Cu^{2+} and Ni^{2+} display a negative visible circular dichroism in their lysine-vasopressin complexes consistent with coordination at the amino terminus and chelation at up to three deprotonated amide nitrogens (section IXB).[303]

The octapeptide Val^5-angiotensin II-Asp^1-β-amide [H·Asp(NH_2)-Arg-Val-Tyr-Val-His-Pro-Phe·OH], which has hypertensive activity, reacts with Cu^{2+} and Ni^{2+} with release of protons. Cu^{2+} forms a blue-violet complex at pH >8 and Ni^{2+} a yellow species at about pH 9.[304] These color changes indicate that ionized amide nitrogens participate in complex formation. The Cu^{2+} angiotensin 1:1 system[304] does not catalyze the dispro-

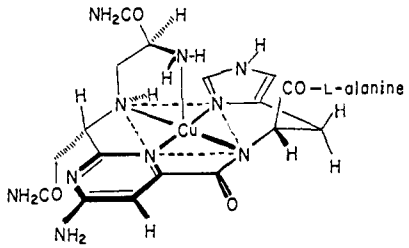
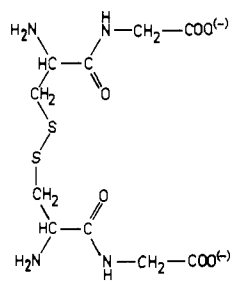


Figure 10. Structure of the Cu^{2+} complex of the bleomycin intermediate P-3A as determined by X-ray diffraction. Reproduced with permission from ref 311. Copyright 1978, Japan Antibiotics Research Association.

portionation of H_2O_2, indicating that the coordination sphere of Cu^{2+} is saturated;[129] there is also no peroxidase-like degradation of the Cu^{2+} complex at pH 8.5.[304] This contrasts with the Ni^{2+} system, which shows some catalase-like activity at pH >8.5;[304] in this system angiotensin II is split by H_2O_2 at pH 10 into characteristic fragments.[304,305] This activity shows that in the Ni^{2+}:angiotensin 1:1 system H_2O_2 or HOO^- finds at least some access to the coordination sphere of Ni^{2+}, because Ni^{2+} with a saturated coordination sphere is catalytically inactive.[306,307]

The antibiotic bleomycin has been isolated as an equimolar Cu^{2+} complex.[308-310] The presence of Cu^{2+} appears essential for biosynthesis. A crystal structure determination of a synthetic intermediate (P-3A) shows Cu^{2+} binding to five nitrogen donors, including, in the equatorial plane, both the deprotonated amide nitrogen and an imidazole nitrogen of a histidine residue (Figure 10).[311] Consistent with the principles developed in this review, coordination at the deprotonated amide nitrogen is anchored; in the case of intermediate P-3A, anchored on both sides of the amide bond. The first anchor is an imidazole nitrogen of the histidine residue (sections IIIC and XI). The histidine nitrogen is amidated by a substituted 4-pyrimidinecarboxylic acid, which should result in considerable acidification of the amide hydrogen, making it easier to deprotonate than a normal peptide hydrogen. The N-3 of pyrimidine also coordinates to Cu^{2+} and serves as a second anchor for the deprotonated amide nitrogen. This anchor is itself anchored by an amino acid side chain from the 2-position of the pyrimidine ring, making an additional 5-membered chelate ring (Figure 10). Solution studies show the liberation of 4 equiv of acid by pH 6 for Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, and Fe^{2+}.[312] Amide deprotonation is implied for all five metal ions. The Fe^{2+} complex of bleomycin is thought to be the physiologically active form.

The disulfide linked small peptide L-cystinylbis(glycinate) (**39**) may be viewed as the two terminal amino ends of a two-chained protein with its two chains linked



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via a disulfide bridge. Potentiometric pH titrations indicate that both terminal end groups of the peptide (**39**) bind Cu^{2+} and amide protons are released;[299,300] the spectral characteristics in the visible range correspond to those obtained for the Cu^{2+} complexes with alanylglycinate and valylglycinate.[299] Hence, a glycylglycinate-like coordination sphere (structure **23**) is suggested. Both mono- and binuclear complexes are formed, and there is also the possibility of a weak (apical) participation of the disulfide linkage in complex formation.[299]

B. Homopolypeptides

In long peptides the single amino terminus, though still serving as an anchor, becomes less significant compared to potential side-chain interactions with metal ions. We distinguish three kinds of complexes of Cu^{2+} and other metal ions with polypeptides. At low pH metal ions interact exclusively with the side chains (type S), at higher pH there is a combined involvement with side chains and deprotonated peptide nitrogens of the backbone (type SP), and finally at pH >10–12 Cu^{2+} interacts exclusively with four deprotonated peptide nitrogens in a biuret-type complex (type B). The mixed type SP complex forms only when a 6-membered chelate ring may form between a side chain donor atom and a deprotonated peptide nitrogen. The strong tetragonality of the Cu^{2+} biuret type B complex makes apical coordination of a side chain group unlikely (section IXA).

With the homopolypeptides of lysine[313,314] and ornithine[313,315] having long, nonchelatable side chains, Cu^{2+} forms type S complexes in neutral solutions (in agreement with the observed catalase-like activity of the Cu^{2+} poly(L-lysine) system)[316] and type B complexes at pH >11. Type SP complexes do not occur. Correspondingly, the Cu^{2+} poly(L-glutamic acid) system has its absorption maximum at about 710 nm, indicating only O-side-chain coordination in the pH range up to 10, whereas at pH >12 a significant blue shift occurs.[316]

On the other hand, homopolypeptides of 2,4-diaminobutyrate[313] and histidine[317] form all three kinds of complexes. Strong chelation of the 2,4-diaminobutyrate side chain in mixed type SP complexes inhibits development of the exclusively type B complex to pH >13.

With polypeptides-containing residues of the L configuration, type S complexes show little optical activity, type SP complexes display a prominent positive shorter wavelength circular dichroism (CD) in the visible region, and type B complexes exhibit a primarily net negative visible CD. CD near 320 nm, characteristic of Cu^{2+} coordination to a deprotonated peptide nitrogen (section IXB)[237] is negative in type SB complexes and tends to be positive in type B complexes. Copolymer polypeptide complexes of Cu^{2+} also follow these trends.[318] Both helical[313] and β secondary[319] structures are destroyed by type B chelation. The precise structure of type SP complexes depends upon the side chain. This topic has been reviewed and some alternative interpretations offered.[156] An NMR study of polylysine failed to detect peptide coordination at high pH with a 200- to 2000-fold side-chain excess over Cu^{2+}.[320] This result is expected and due to the inability of chelate rings containing deprotonated peptide nitrogens to close

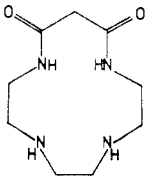
before a Cu^{2+} passes onto another side chain[108,110] (section IVA5).

XIV. Cyclic Ligands with Amide Functions

Such ligands permit study of metal ion interaction with an amide linkage and with side-chain substituents under conditions where effects of the terminal functional groups of linear peptides are excluded.

A. Macrocycles with Amino and Amide Nitrogens

The recent synthesis[321] of the 13- to 15-membered macrocyclic dioxo tetraamines, 1,4,7,10-tetraazacyclotridecane-11,13-dione (**40**), 1,4,8,11-tetraazacyclotetradecane-12,14-dione (**41**), and 1,4,8,12-tetraazacyclopentadecane-9,11-dione (**42**), led to the study of their



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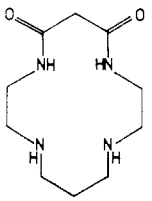

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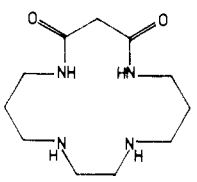


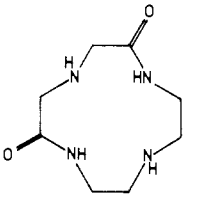
42

Cu^{2+} complexes.[322] All three ligands form the neutral amide ionized Cu(L-2H) species; a monodeprotonated $Cu(L\text{-}H)^+$ complex was *not* observed. In substitution reactions the Cu^{2+} complex of the 14-membered macrocycle **41** is the most inert.[323] The same complex is also the most stable among the three complexes:[322] in potentiometric pH titrations, beginning with H_2L^{2+}, all four protons are liberated before pH 5 is reached; this complex of **41** is also much more stable than the corresponding species of the open-chain diamide *N,N'*-bis(2-aminoethyl)malondiamide (section XVIA2)[322] though the absorption maxima of both complexes at 506 and 516 nm are relatively similar.[324] These absorption maxima confirm the coordination of two ionized amide nitrogens in both complexes (section IXA). With Zn^{2+}, Cd^{2+}, and Pb^{2+} the dioxo ligands **40–42** underwent no deprotonation at pH <7, and at pH >7 precipitation of the hydrolyzed metal ions occurred.[322]

Of the 14-membered ligand **41** the neutral M(L-2H) complexes with Ni^{2+} and Cu^{2+} have been prepared, and the brick red *trans*-$[Co^{III}(L\text{-}2H)(NH_3)_2]Cl$ complex has also been isolated.[324] In ethanolic solution the amide-ionized $Co^{II}(L\text{-}2H)$ forms a reversible adduct with O_2 at −70 °C in the presence of imidazole or pyridine.[325] The Ni(L-2H) complex is orange-yellow and diamagnetic;[324] in dimethyl sulfoxide this complex may be methylated by CH_3I in the presence of suspended pulverized KOH.[326] Under these strongly alkaline conditions methylation occurs at the coordinated amino nitrogens and also at the methylene group of the malonamide unit of **41** without attacking and destroying the amide linkage. Obviously, Ni^{2+} is protecting the amide group and also activating the methylene group for C-alkylation (section VIIA).

The Ni(L-2H) and Cu(L-2H) complexes of the macrocycle **41** are acid labile, unlike the analogues of 1,4,8,11-tetraazacyclotetradecane (cyclam);[324] this is due to the possibility in the complexes of **41** of protonating the oxygen of the coordinated ionized amide groups (section IIB). The Ni^{2+} and the Cu^{2+} complexes of **41** may also be oxidized to $Ni^{III}(L\text{-}2H)^+$ and $Cu^{III}(L\text{-}2H)^+$;[327] the greater stability of the trivalent state of copper in $Cu(L\text{-}2H)^+$ compared with $Cu(cyclam)^{3+}$ is in agreement with the conclusions in section VIII: peptide N^- stabilizes Cu^{3+} better than an amino nitrogen.

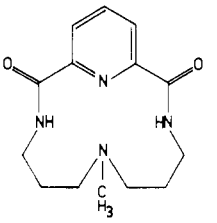
The 12-membered macrocyclic ligand 1,4,7,10-tetraazacyclododecane-2,6-dione (**43**), which contains in



43

its cycle two glycyl units, forms with Ni^{2+} and Cu^{2+} the amide-ionized species M(L-2H).[328] The stability of these complexes is much smaller than that of the corresponding complexes with the larger macrocycles **40–42**, and in the case of Cu^{2+} the monoionized complex $Cu(L\text{-}H)^+$ was also observed. In agreement with the small cavity size $Cu^{II}(L\text{-}2H)$ is easily oxidized to $Cu^{III}(L\text{-}2H)^+$. Similarly, the yellow, low-spin, planar Ni(L-2H) complex may also be oxidized to its Ni^{3+} analogue.

The pyridyl-containing 14-membered macrocyclic ligand 44 forms with Cu^{2+} the complex $Cu(L)^{2+}$, which



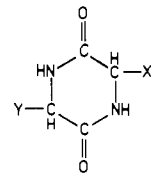
44

is reported as being yellow in the crystalline form and red-violet in methanol solution;[329,330] infrared measurements with KBr pellets confirm that in the solid $Cu(L)Cl_2$ the amide group is not ionized.[330] It appears that the color change of this complex in dissolving is due to a structural rearrangement connected with an intramolecular acid–base reaction, i.e., transfer of an amide proton to the amino nitrogen. The addition of 2 equiv of base results in the amide-ionized neutral complex Cu(L-2H); this is confirmed by the absorption maximum of 512 nm and infrared measurements.[330]

In summary: from the results obtained with the macrocyclic ligands **40–44** it is evident that amino donor atoms within the cycle can act as primary ligating groups (section III). This leads then to complexes in which the metal ions are incorporated into the ring system during deprotonation and simultaneous coordination of the amide nitrogens.

B. Cyclic Peptides

In this section we consider first some cyclic dipeptides before progressing to macrocyclic peptides. Several cyclic dipeptides, i.e., substituted 2,5-piperazinediones,



45

45 a: cyclo-L-His-L-His X = $-CH_2$-(imidazole) = Y

b: cyclo-Gly-L-His = $-CH_2$-(imidazole) Y = H

c: cyclo-Gly-L-Asp = $-CH_2-COO^-$ = H

d: cyclo-Gly-L-Glu = $-CH_2-CH_2-COO^-$ = H

e: cyclo-Gly-L-Ser = $-CH_2-OH$ = H

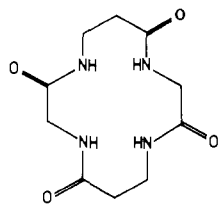
f: cyclo-Gly-L-Asn = $-CH_2-CONH_2$ = H

g: cyclo-Gly-L-Gln = $-CH_2-CH_2-CONH_2$ = H

are summarized under structure **45**. From these ligands *cyclo*(L-His-L-His) (**45a**) has received the most attention. The crystal structure study[331] of the cationic Cu^{2+} complex $[(H_2O)Cu_2(cyclo(\text{L-His-L-His}))_2]^{2+}$ revealed a dimer with each of the two *cyclo*(L-histidyl-L-histidyl) ligands bridging the two Cu^{2+} ions. One Cu^{2+} is five-coordinate with donor atoms from a water plus an imidazole nitrogen and an amide carbonyl oxygen from each of the two ligands in the dimer. The other Cu^{2+} adopts a flattened tetrahedral geometry with donor atoms from an imidazole nitrogen and a deprotonated amide nitrogen from each of the two ligands. Of the two amide bonds in each ligand one is not involved in coordination while the other is doubly coordinated: the carbonyl oxygen to one Cu^{2+} and the deprotonated amide nitrogen to the other. Some electronic properties of this complex have been investigated.[332] An X-ray structure analysis of the monomeric $Cu(cyclo\text{-(L-His-L-His)})_2(ClO_4)_2 \cdot 4H_2O$ complex has also been performed;[333] Cu^{2+} is in this case tetrahedrally distorted and coordinated only to the four imidazole residues of the two ligands without involvement of the amide groups. Studies of this complex in aqueous solution have also been done,[334] and the stability constants of the corresponding complexes with Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} were determined.[335]

Cu^{2+} substitution for an amide hydrogen in the cyclic dipeptides appearing in structures **45b–g** is less favorable than in their linear counterparts and occurs, if at all, well beyond the physiological pH range.[336] Even where a potential anchor exists in ligands **45b–d**, amide hydrogen ionization does not occur until pH >10. With compounds **45e–g** there is no hydrogen ionization at pH <11.6. Base hydrolysis of the diketopiperazine ring is inhibited by Cu^{2+}. Solvolysis of the "dangling" amide residue of the two peptides *cyclo*(Gly-L-Asn) and *cyclo*(Gly-L-Gln) is selectively promoted by Cu^{2+} in aqueous alkali to yield complexes identical with those found with *cyclo*(Gly-L-Asp) and *cyclo*(Gly-L-Glu).

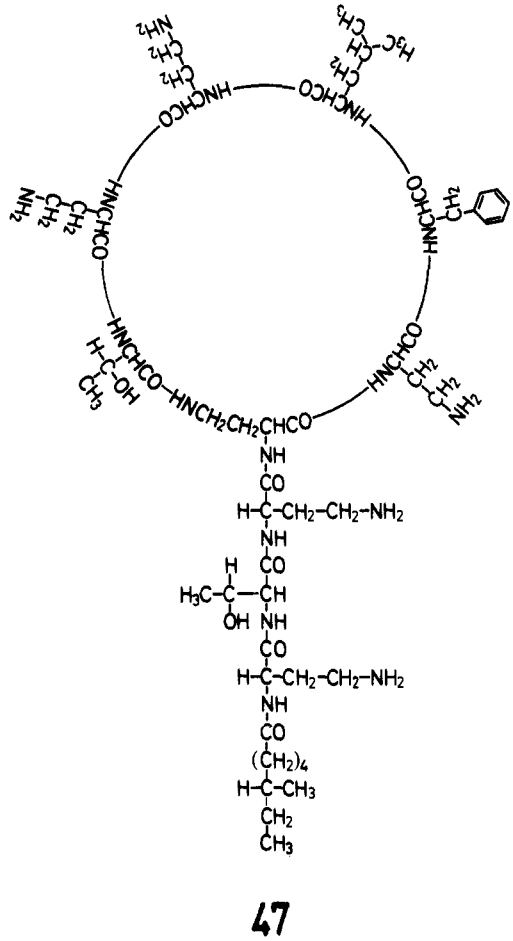
The 14-membered macrocyclic tetrapeptide *cyclo*(β-alanylglycyl-β-alanylglycyl) (**46**) reacts with Cu^{2+} to



46

form a quadruply deprotonated peptide complex with alternating 5- and 6-membered rings.[337] As the tetrapeptide **46** has no terminal groups that might function as primary ligating sites, it is not surprising that rather drastic conditions are needed to form the $Cu(L\text{-}4H)^{2-}$ complex: a slurry of the ligand must be treated with freshly precipitated copper(II) hydroxide at pH >13; the resulting complex is stable in the pH range down to 8. The $Cu(L\text{-}4H)^{2-}$ complex is square planar, with an absorption maximum at 488 nm; this maximum remains unaltered in the pH range 8.2–14. This Cu^{2+} complex is, as expected (section VIII), easily oxidized to $Cu^{III}(L\text{-}4H)^-$, and this Cu^{3+} complex is relatively stable in neutral solution with a half-life of 5.7 weeks at 25 °C, which may be compared with the half-life of 5.5 h of the Cu^{3+}–tetraglycine complex $Cu(GGGG\text{-}3H)^-$.[338] The decomposition of $Cu^{III}(L\text{-}4H)^-$ leads to Cu^{2+} and oxidized ligand; the reaction is acid and base catalyzed. Both complexes, $Cu^{II}(L\text{-}4H)^{2-}$ (ref 337) and $Cu^{III}(L\text{-}4H)^-$ (ref 338), are thermodynamically and kinetically more stable than the corresponding complexes with linear peptides.[234]

The antibiotic polymyxin B_1 (47) consists of a cyclic



47

heptapeptide with a tripeptide residue in a side chain; the terminal function of this side chain is (+)-6-methyloctanoic acid (isopelargonic acid) in polymyxin B_1 (47) and isooctanoic acid (6-methylheptanoic acid) in polymyxin B_2.[339] Potentiometric pH titrations of the Cu^{2+}–polymyxin B system showed that Cu^{2+} releases four protons in the pH range up to 8.5; the structure proposed for this complex consists of a 5- and a 6-membered chelate ring formed between two neighboring ionized amide nitrogens and the γ-amino group of an

α,γ-diaminobutyric acid link within the cyclic heptapeptide part.[340] It was further proposed that the fourth equatorial position at Cu^{2+} is occupied by an amino group via a macrochelate.[340] That the equatorial positions of this Cu^{2+}–polymyxin B complex are largely saturated is confirmed by the relatively low catalase-like activity of this complex.[341]

The corresponding Ni^{2+} complex, formed at pH 9, is yellow, with an absorption maximum at 437 nm, indicating planar coordination of Ni^{2+} with participation of ionized amide nitrogens.[340] On the other hand, this Ni^{2+}–polymyxin B (1:1) system shows a catalase-like activity in the pH range >7, indicating that H_2O_2 or HOO^- have some access to the coordination sphere of the metal ion.[306] But at the same time the Ni^{2+}–polymyxin B complex has at pH 9.4 a very specific structure: its ORD spectrum was important during the synthesis of polymyxin B_1 and in proving the identity of the synthetic and natural products.[339,342] The Cu^{2+} and the Ni^{2+} complexes of polymyxin B may be selectively degraded with hydrogen peroxide at pH 8.5 and 9.4, respectively.[343] At pH >9 polymyxin B can coordinate a second Cu^{2+}, and clear solutions are obtained;[340] this system shows a significant catalase-like activity, indicating that the equatorial coordination sphere of at least one of the two Cu^{2+} is not saturated.[341] During potentiometric pH titration of a 1:1 system with Co^{2+} the formation of a precipitate was observed at pH 8.2, indicating that no stable Co^{2+}–polymyxin B complex is formed.[340]

Several bicyclic decapeptides have been synthesized; the two rings being connected via an S–S bridge.[344,345] These cyclic peptides show some cation specificity within the alkali and alkaline earth cations; the coordination occurring via the carbonyl oxygens of the amide linkages. Similar observations have been made with several naturally occurring cyclopeptides, including valinomycin and enniatin B, as summarized in a recent review.[346] The synthetic heptapeptide, *cyclo*(Gly-L-His-Gly-L-His-Gly-L-His-Gly),[347] and the octapeptide, *cyclo*(Gly-L-Glu-Gly-Gly-L-His-Gly-L-His-Gly),[348] complex Zn^{2+} via three imidazole residues, and two imidazole and one Glu-carboxylate group, respectively; in the ^1H NMR spectrum all the peptide NH resonances were observable and unaffected, indicating that the amide nitrogens are not involved in metal ion binding under the used conditions (Me_2SO-d_6 solvent).

XV. Proteins

Looking back at Table I, we note that in solutions of tetraglycine by pH 9 two peptide hydrogens are deprotonated by Cu^{2+} and three by Ni^{2+}. To the extent that tetraglycine serves as a model ligand for the amino terminal four amino acid residues, we anticipate similar peptide deprotonations in proteins. Proteins with an acetylated amino terminus or a proline among the second to fourth residues would, of course, be unable to bind metal ions in the manner of tetraglycine. A chelated complex with deprotonated peptide nitrogens is also incompatible with an α helix, which, if it did occur at the amino terminus, would have to unwind to become chelated. At more nearly neutral pH values, interaction of Cu^{2+} and Ni^{2+} at the amino terminus of proteins is incomplete and should suffer competition from imidazole and other side chains elsewhere on the molecule.

Deduced from studies on peptides, these conclusions are borne out when Cu^{2+} or Ni^{2+} is added to many proteins. Addition to α-chymotrypsin and ribonuclease of Cu^{2+} near pH 8 or Ni^{2+} near pH 10 produces a negative peak in the visible circular dichroism spectra similar to that exhibited by peptides that do not contain histidine (section IXB).[303] These results suggest interaction of the metal ion at the amino terminus of the proteins. In the same pH range, however, interactions also occur at histidyl residues. Neutral solutions of ribonuclease contain five Cu^{2+} binding sites, and distinguishing among them remains difficult. The system has received both short[349] and long[350] reviews. At high pH about 30 Cu^{2+} react with ribonuclease to form biuret-type complexes with four deprotonated amide nitrogens about each Cu^{2+}.

Results reviewed for histidine peptides in section XI suggest that if a histidyl residue resides in the second or third position from the amino terminus, an especially strong interaction with Cu^{2+} and Ni^{2+} becomes complete by neutral solutions. Albumins consist of single polypeptide chains with molecular weights about 65 000. Reports that bovine serum albumin (BSA) binds relatively strongly 1 mol of Cu^{2+}/mol of protein at a site other than the lone sulfhydryl group led to the suggestion that the first equivalent of Cu^{2+} is bound at the amino terminal aspartyl residue.[351] Cu^{2+} binding to BSA is stronger than to amino acid aspartate and is inhibited by Ni^{2+} but not by Zn^{2+}.[351] Direct experiments with Ni^{2+} showed that an equimolar solution with BSA yields the same yellow color and absorption maximum from 410 to 450 nm observed in small peptide complexes. As a result it was suggested that the first equivalent of Cu^{2+} and Ni^{2+} reacts not merely with the amino terminal residue of albumins but with the amino nitrogen and with chelation at succeeding deprotonated peptide nitrogens.[352] A yellow absorption band was also observed upon addition of Ni^{2+} to human serum albumin (HSA) which possesses the amino terminal sequence Asp-Ala-His-, and it was also suggested that the histidyl residue in the third position made interaction at the amino group and two deprotonated peptide nitrogens more likely.[352]

Subsequent studies concentrated on the interaction of the first equivalent of Cu^{2+} with BSA. Proton displacement upon addition of Cu^{2+} to BSA and the similarity of the visible absorption spectrum to that observed with Cu^{2+} peptides after peptide ionization suggested that two peptide nitrogens are coordinated in addition to the α-amino nitrogen.[353] Participation of the histidyl residue in Cu^{2+} binding to BSA was advanced on the basis of studies with the amino terminal 24-residue peptide fragment of BSA,[354] which contains the amino terminal sequence Asp-Thr-His-Lys.[355] This tetrapeptide, the 24-residue peptide, and BSA on binding of 1 equiv of Cu^{2+} all yield similar proton displacements plus visible absorption and circular dichroism spectra.[356] Binding of 1 equiv of Cu^{2+} to the 24-residue peptide fragment protects the α-amino group and the histidyl residue in position 3 from carboxymethylation. Thus the inference is strong that the first equivalent of Cu^{2+} added to BSA binds to an α-amino nitrogen, two intervening ionized peptide nitrogens, and an imidazole nitrogen from the histidyl residue in position 3. A second equivalent of Cu^{2+} appears to

complex about equally with two interior histidyl residues of the 24-residue peptide fragment.[356]

Visible circular dichroism (CD) results support involvement of a histidyl residue in the amino terminal interaction of Cu^{2+} and Ni^{2+} with BSA. Rather than the uniformly negative CD such as occurs upon their interaction with ribonuclease (above), the first equivalent of Cu^{2+} and Ni^{2+} reacts with BSA to yield the shorter wavelength positive and longer wavelength negative CD characteristic of a histidyl residue interaction (section IXB2).[303,355,356]

Albumins from humans, cows, and rats all possess a histidyl residue in the third position, and all three strongly bind 1 equiv of Cu^{2+} in neutral solutions. Dog albumin contains a tyrosyl residue in the critical third position and binds Cu^{2+} weakly.[357,358] In contrast to the biphasic positive and negative CD with BSA and HSA, addition of 1 equiv of Cu^{2+} to dog albumin yields only a uniformly negative visible CD, indicating no histidyl residue involvement.[349]

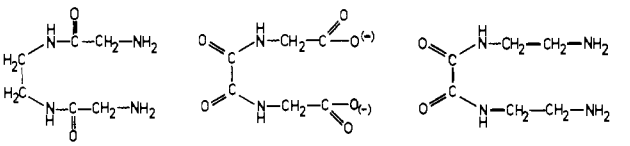
The Cu^{2+} binding properties of the tripeptide, L-Asp-L-Ala-L-His-*N*-methylamide, the sequence corresponding to the amino terminus of HSA, are similar to those of HSA.[358,359] Kinetic experiments indicate comparable behavior on the dissociation of Cu^{2+} from BSA[360] and Gly-Gly-His[361] that differs from that for non-histidyl-containing peptides.

Two groups of investigators have interpreted results from vibrational spectroscopy to indicate binding of Cu^{2+} to deprotonated peptide nitrogens in small, intensely blue copper proteins such as plastocyanin and azurin. For plastocyanin both resonance Raman[362] and infrared[363] results were interpreted as being due to deprotonated peptide nitrogen coordination. The infrared study also indicated similar binding by Co^{2+} in the Co^{2+}-substituted plastocyanin. We have already noted in section IVF, however, that it is about 10^6 times more difficult for Co^{2+} than for Cu^{2+} to deprotonate a peptide nitrogen. For the Cu^{2+} proteins absorption bands in the visible were assigned to deprotonated amide to Cu^{2+} charge-transfer transitions.[364] There is no evidence for such low-energy charge-transfer transitions in any other deprotonated amide–Cu^{2+} complex (section IXA).[237] It was also suggested that the deprotonated peptide nitrogen is a few residues removed from a histidine with a coordinated imidazole side chain;[364] there is no nearby anchor for the coodinated peptide nitrogen. Similar Cu^{2+} binding to a deprotonated peptide nitrogen has been suggested for other blue copper proteins including azurin.[364] Crystal-structure determinations of plastocyanin[365] and azurin[366] reveal proteins of similar overall structure with four donor atoms to a distorted tetrahedral Cu^{2+} from side chains of two histidine, one cysteine, and one methionine residues. There is no deprotonated peptide nitrogen coordination. Thus interpretations from vibrational spectroscopy concerning amide bond coordination are again faulty, as already noted for protonation (section IIB) and metalation (section IVF).

XVI. Selected Examples of Other Amides

A. Diamides

The ligands shown in structures **48**–**50** are related to each other and to small peptides as well; all three ligands contain two amide groups. A discussion of their comparative coordinating properties, including also

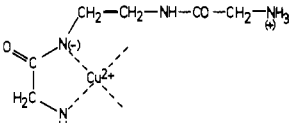


48 49 50

several related ligands, is now in order.

1. Ethylenediamine Derivatives and Related Ligands

From a recent detailed study[367] using a combination of potentiometric and spectrophotometric measurements it is known that *N*,*N*′-diglycylethylenediamine (**48**) forms with Cu^{2+} the complexes $Cu(HL)^{3+}$ and $Cu(HL)_2^{4+}$ in which the ligand is coordinated via a terminal amino group and the oxygen of the neighboring amide moiety. The complex $Cu(HL)^{3+}$ loses a proton with $pK_a = 5.78$; the resulting complex $Cu(L)^{2+}$ may be an isomeric mixture between $Cu(HL)(OH)^{2+}$ and the species shown in structure **51**, but the absorption measurements indicate that **51** dominates. Complexes like $Cu(L)(HL)^{3+}$ or $Cu(L)_2$ with neutral amino end groups are not observed. The complex $Cu(L)^{2+}$ (**51**) loses two protons at pH ~7.0 in a single step ($pK^{2H}_{CuL} = 14.05$) to form Cu(L-2H), which has structure **52** as



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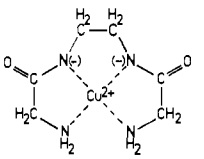
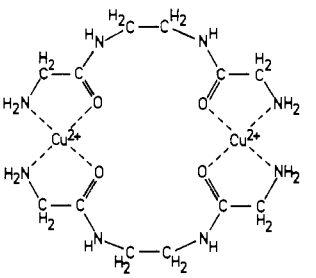


52

is evident from the absorption maximum at 513 nm. However, the experimental data cannot be solely explained with the mentioned complexes: the dimeric species $[Cu(L)]_2^{4+}$ and $[Cu(L\text{-}H)]_2^{2+}$ are formed in addition;[367] actually the latter complex had been postulated already some time ago.[368] To these complexes structures **53** and **54** have now been assigned on the basis of absorption measurements.[367]



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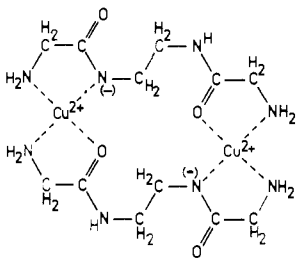


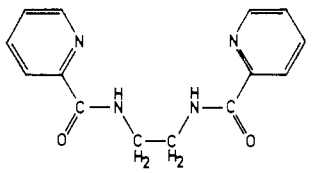
54

The number of methylene groups between the two amide nitrogens in structure **52** influences the coordinating properties: 1,3-bis(glycylamino)propane forms the complex analogous to structure **52** at pH about 5.5; the second ammonium proton and the second amide proton are again lost in a single step, but now with $pK^{2H}_{CuL} = 10.91$.[367] This value shows that the species Cu(L-2H) of 1,3-bis(glycylamino)propane is by about 3 log units more stable than the corresponding one of N,N'-diglycylethylenediamine (**48**) ($pK^{2H}_{CuL} = 14.05$). This larger stability of Cu(L-2H) is most probably the reason with 1,3-bis(glycylamino)propane only the dimer $[Cu(L)]_2^{4+}$ is observed, i.e., no indication for $[Cu(L-H)]_2^{2+}$ was found. The larger stability of Cu(L-2H) of 1,3-bis(glycylamino)propane indicates further that the ethylene bridge in **52** is strained and that the replacement of this short bridge by the longer propylene bridge releases this strain. Of course, additional lengthening of this bridge will not further encourage complex formation: with 1,5-bis(glycylamino)pentane[369] the "primary" complex $Cu(HL)^{3+}$ releases its first amide proton with $pK_a = 5.49$ to form a species with a structure analogous to **51**. The ammonium group is then deprotonated at $pK_a = 7.47$ but does not coordinate;[369] a complex analogous to structure **52** forms only after deprotonation of the second amide group at $pK_a = 10.30$.

The described structures agree well with the catalytic properties of these Cu^{2+} complexes in the promotion of the disproportionation of H_2O_2;[129,341] in these reactions the substrate is coordinated, and activity is therefore only observed as long as accessible equatorial binding sites exist at Cu^{2+}. In agreement herewith the Cu^{2+}–1,5-bis(glycylamino)pentane system has a pronounced catalase-like activity in the pH range above 6, while the Cu^{2+}–1,3-bis(glycylamino)propane system (due to the formation of Cu(L-2H) with a structure analogous to **52**) is inactive from pH 5 to 11. Most fascinating is the fact that the Cu^{2+}–*N,N'*-diglycylethylenediamine system has a catalase-like activity in the pH range 6.5–9.5 with a maximal activity at pH 8 due to the formation of the species $Cu(HL)^{3+}$ and $Cu(L)^{2+}$ (**51**). With the formation of the fourfold coordinated complex Cu(L-2H) (**52**) the catalase-like activity decreases, and at pH about 10 the system is inactive.

N,N'-diglycylethylenediamine (**48**)[370,371] and 1,3-bis(glycylamino)propane[371] form with Ni^{2+} a planar complex which has a structure analogous to **52**,[371] while Co^{2+} and Zn^{2+} form only rather weak complexes with the neutral *N,N'*-diglycylethylenediamine.[368] Related to the latter ligand (**48**) is *N,N'*-ethylenedi-2-pyridinecarboxamide (**55**) which gives with Cu^{2+} the neutral Cu(L-2H) complex with ionized amide nitrogens as is revealed by a crystal structure analysis.[372]

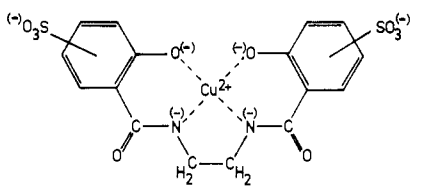
Replacement of the ethylene bridge in **55** by a 1,2-



55

cyclohexane unit gives the ligand *N,N'*-1,2-cyclohexanediylbis(2-pyridinecarboxamide), and from its Cu^{2+} complex a crystal structure determination[373] was carried out: again both ionized amide nitrogens and both pyridine groups are coordinated. The corresponding complexes with Ni^{2+}, Pd^{2+}, and Pt^{2+} have also been isolated; all are neutral, yellow, and diamagnetic, and a planar metal atom environment is suggested.[374] With Zn^{2+} and Co^{2+} only the cationic $M(L)^{2+}$ complexes were isolated as the $Zn(L)I_2$ and $Co(L)(SCN)_2$ salts.[374] The ligand *N,N'*-o-phenylenebis(2-pyridinecarboxamide) with a 1,2-benzene unit instead of the ethylene bridge in **55** also forms a quadridentate ligand with two pyridine nitrogens and two deprotonated amide nitrogens coordinated to Cu^{2+} (structure **13**; section IIIB);[43] in this case the neutral complexes of Co^{2+}, Ni^{2+}, Zn^{2+}, and Pd^{2+} were also isolated and a planar $M^{II}(N)_2(N^-)_2$ system is proposed for these complexes.[375]

Substitution of the two pyridyl groups in the diamide ligand **55** by sulfonated phenol residues gives a ligand, which in the presence of Cu^{2+} upon the addition of base liberates 4 equiv of protons, leading to a red-violet complex for which structure **56** was suggested.[376] With

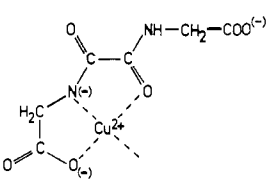


56

the analogous ligands, in which the ethylene bridge is replaced by propylene or butylene units, no complex corresponding to **56** was observed.[376] These observations are meaningful regarding the discussion at the end of section IIIC about the possibility that phenol groups could serve as primary ligating groups; it appears now that this is possible, but only under especially favored structural conditions. There are also incomplete hints[376] that V^{3+} might replace amide protons in such phenol–diamide ligands.

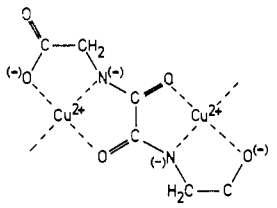
2. Oxalamide Derivatives and Related Ligands

Oxalamide-*N,N'*-diacetate (**49**) forms with Co^{2+}, Zn^{2+}, and Cd^{2+} weak complexes of the probable composition M(L) while more stable complexes are formed with Cu^{2+} and Ni^{2+} by ionization of the amide group(s).[377] With excess ligand in weakly acidic solution (pH $\simeq$ 5) a 1:1 complex, for which structure **57** was suggested, is



57

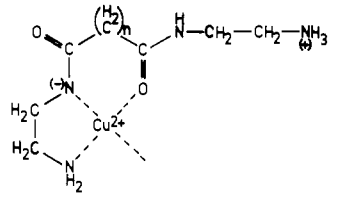
formed; this $Cu(L\text{-}H)^-$ species, in the presence of excess Cu^{2+} (pH $\simeq$ 5), can add another Cu^{2+}, leading to the neutral binuclear complex $Cu_2(L\text{-}2H)$, for which structure **58** was suggested. Under conditions with excess



58

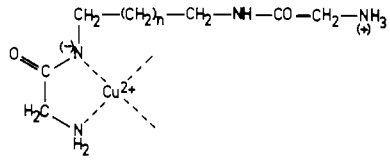
ligand also a species $Cu(L\text{-}H)_2^{4-}$ is formed. As a result, in contrast to *N,N'*-diglycylethylenediamine (**48**, section XVIA1), the ligand oxalamide-*N,N'*-diacetate (**49**) does not form a 1:1 complex in which both amide groups are deprotonated with Cu^{2+} (or Ni^{2+}), and hence a complex analogous to structure **52** does not occur.

N,N'-Bis(2-aminoethyl)oxalamide (**50**) and *N,N'*-bis(2-aminoethyl)malonamide in the presence of Cu^{2+} at pH about 6 form the species $Cu(L)^{2+}$, with the simultaneous release of two protons; the structure **59** (n = 0, 1) was proposed[378] in accordance with spectro-



59

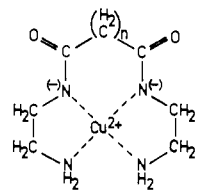
photometric measurements.[379] Complexes analogous to **59**, where $n \geq 2$, are not stable: hydrolysis and precipitation of $Cu(OH)_2$ are favored in these cases.[378] However, the homologous ligands of *N,N'*-diglycylethylenediamine (**48**) form Cu^{2+} complexes of structure **60**) for all the known values of n, i.e., n = 0–4, 6 (section



60

XVIA1).[367,369] Thus, the chelate **60** is more stable than **59**, probably as the result of the endocyclic carbonyl group in **60**, which occupies an exocyclic position in **59**.

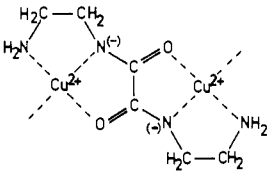
At pH >7 the complexes with structure **59**, n = 0 or 1, release an additional proton,[378] and there are indications that this release results from hydroxo complex formation.[379] Above pH 7.5 the neutral species Cu(L-2H) forms; structure **61** (n = 0 or 1) is attributed to this species.[378] The neutral Cu^{2+} and Ni^{2+} complexes



61

of the malondiamide derivative **61** (n = 1) were isolated, and the Ni^{2+} complex is yellow and diamagnetic as expected; with cobalt the diamagnetic complex $[Co^{III}(L\text{-}2H)(NH_3)_2]Cl$ was obtained.[380] It is evident from the study of the Cu^{2+} complexes in solution[378] and from the corresponding complexes with *N,N'*-1,3-bis(glycylamino)propane (analogous to **48**; section XVIA)[367,369] that the species Cu(L-2H) are especially stable if the two coordinated and ionized amide nitrogens are linked by three carbon atoms, which allows the formation of a 6-membered ring (structure analogous to **52**, and structure **61** with n = 1).

However with *N,N'*-bis(2-aminoethyl)oxalamide (**50**) additional evidence was given[378] that a binuclear complex $Cu_2(L\text{-}2H)^{2+}$ is formed to which structure **62** is



62

attributed; this is similar to structure **58**. The corresponding complex $Cu_2(L\text{-}2H)^{2+}$ with *N,N'*-bis(2-aminoethyl)malondiamide was not observed; this result indicates that the formation of these binuclear species demands the planar oxalamide moiety. Such an indication agrees with the observations made with *N,N'*-bis(2-pyridylmethyl)oxalamide,[378,381] which also forms the binuclear species $Cu_2(L\text{-}2H)^{2+}$, again this species is not formed with the corresponding malonamide derivative.[378,381]

The coordinating properties of *N,N'*-bis[2-(dimethylamino)ethyl] oxalamide[379] closely resemble those described for the Cu^{2+}–*N,N'*-bis(2-aminoethyl)oxalamide system;[378] again the complexes corresponding to structures **59** and **62** have been shown to occur. Not only can hydroxo ligands be coordinated[379] by the corresponding complexes $Cu(L)^{2+}$ (analogous to **59** with n = 1) and $Cu_2(L\text{-}2H)^{2+}$ (analogous to **62**), but also mixed ligand complexes may be formed with SCN^-, CN^-, SO_3^{2-}, or imidazole.[382] For the dithiocyanate complex, $Cu_2(L\text{-}2H)(SCN)_2$, the structure analogous to **62** was confirmed by X-ray analysis.[383] Since in both species, $Cu(L)^{2+}$ and $Cu_2(L\text{-}2H)^{2+}$, accessible coordinating sites exist at the equatorial positions of Cu^{2+}, the formation of ternary complexes is not surprising. The ternary complex formed between $Cu(L)^{2+}$ of *N,N'*-bis-[2-dimethylamino)ethyl]oxalamide (its structure is analogous to **59** with n = 1)[379] and imidazole is more stable than the corresponding binary Cu(imidazole)$^{2+}$ complex. Hence, $\Delta \log K_{Cu}$ is a positive value (cf. section VIA; i.e.,

$$\Delta \log K_{Cu} = \log K^{Cu(L)}{}_{Cu(L)(Im)} - \log K^{Cu}{}_{Cu(Im)}$$
$$= 4.6 \text{ (ref 382)} - 4.22 \text{ (ref 192)} = +0.4$$

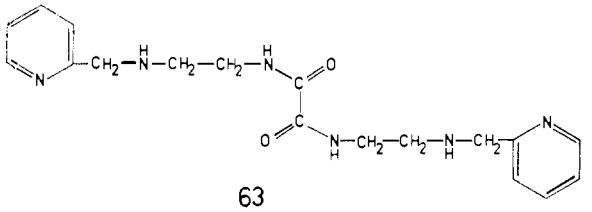
and therefore the equilibrium

$$Cu(L)^{2+} + Cu(Im)^{2+} \rightleftharpoons Cu(L)(Im)^{2+} + Cu^{2+}$$

is displaced to the right in accord with other observations for similar mixed-ligand complexes.[35,36,192,384]

The results described for the ligands **48**–**50** have initiated further studies, namely of the Cu^{2+} and Ni^{2+} complexes of 3,7-diazanonanediamide and its diethyl

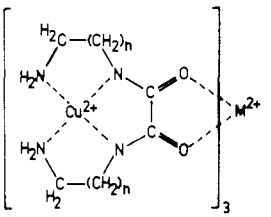
derivative, *N,N'*-diethyl-3,7-diazanonanediamide,[385] as well as of the ligand *N,N'*-bis-[2-[2-(pyridylmethyl)-amino]ethyl]oxalamide, which has structure **63**.[386]



63

Comparable to the binuclear Cu^{2+} complex with structure **62**, ligand **63** forms in aqueous solution the binuclear and heteronuclear complexes $Cu_2(L)^{4+}$, $Cu_2(L\text{-}2H)^{2+}$, $Zn_2(L\text{-}2H)^{2+}$, $Cu(L\text{-}2H)Ni^{2+}$, and probably also $Cu(L\text{-}2H)Zn^{2+}$,[386] as shown by potentiometric pH titrations and spectrophotometric measurements.

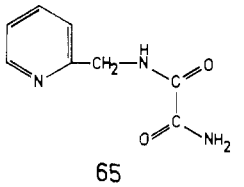
Even heterometal tetranuclear complexes of the type $M^{II}[Cu^{II}(L\text{-}2H)]_3(ClO_4)_2$ or $(NO_3)_2$, where $M^{II} = Co^{2+}$ or Ni^{2+}, could be isolated, and the general structure **64**



64

was proposed for these species.[387] For *N,N'*-bis(2-aminoethyl)oxalamide (**50**) as ligand the Ni^{2+} complex was obtained, and for *N,N'*-bis(3-aminopropyl)oxalamide the Co^{2+} and Ni^{2+} hetero tetranuclear species were prepared. Cryomagnetic measurements showed that a considerably strong antiferromagnetic spin-exchange interaction is operating between Cu^{2+} and Co^{2+} or Ni^{2+}, i.e., via the oxalamide unit.[387]

The results for the tridentate oxalamide derivatives[388] *N*-(2-aminoethyl)oxalamide, *N*-[2-(dimethylamino)-ethyl]oxalamide, and *N*-(2-pyridylmethyl)oxalamide agree with those summarized here for the tetradentate derivatives. *N*-(2-Pyridylmethyl)oxalamide (structure **65**) forms, however, with Co^{2+} in the pH range 8–9 a

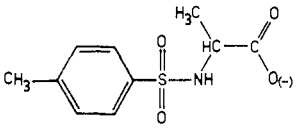


65

green 1:2 complex, $Co(L\text{-}H)_2$,[389] for which a five-coordinated Co^{2+} in a distorted trigonal-bipyramidal environment was deduced.[390] The Co^{2+}–*N*-(2-pyridylmethyl)oxalamide system is also able to form O_2 adducts; complexes, e.g., of the type $[(L\text{-}2H)Co(\mu\text{-}O_2)(\mu\text{-}OH)Co(L\text{-}2H)]^-$ were suggested.[391]

B. Sulfonamides

The amide hydrogen of sulfonamides is more acidic than a peptide hydrogen, and chelation with a sulfonamide nitrogen is possible in appropriate ligands. In aqueous solutions $pK_a = 12.1$ for amide hydrogen ionization in *N*-tosyl-L-α-alaninate (**66**).[392] In the presence

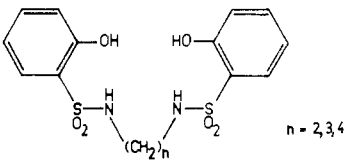


66

of Cu^{2+}, a total of two protons are released by pH 6, and it has been suggested that Cu(L-H) contains a 5-membered chelate ring involving the carboxylate oxygen and deprotonated sulfonamide nitrogen donor atoms. A subsequent deprotonation upon addition of a third equivalent of base with $pK_a = 7.1$ was assigned to hydroxo complex formation from coordinated water.[392] A disquieting aspect of the analysis is the pronounced shift in the visible absorption maximum from 740 to 650 nm that accompanies the $pK_a = 7.1$ deprotonation. Such a large blue shift is not accommodated by water deprotonation and suggests instead substitution of a nitrogen for oxygen donor atom to Cu^{2+} (section IXA). Probably Cu^{2+} substitution for the amide proton occurs with $pK_a = 7.1$.

An analysis of the first two equivalents in the titration curve for *N*-tosyl-α-glycinate indicates a Cu^{2+} stability constant $\log K^{Cu}_{CuL} = 2.2$, suggesting coordination at the carboxylate and only weak chelation at a sulfonamide oxygen; this is accompanied by hydroxo complex formation with $pK_a = 4.9$.[51] Metal ion substitution of the amide proton occurs only for Cu^{2+} with $pK_a = 7.1$ and $PdCl_4^{2-}$ with $pK_a \simeq 5$.[51] Co^{2+}, Ni^{2+}, and Zn^{2+} are ineffective in promoting the amide deprotonation.[392] In line with section III the Cu^{2+}-promoted amide deprotonation occurs only with ligands bearing a primary ligating group.

The bis(sulfonamide) ligands shown in structure **67**



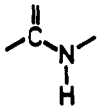
67

liberate four protons in potentiometric pH titrations in the presence of Cu^{2+}, which is indicative of a quadridentate complex formation involving the two phenolate and two deprotonated sulfonamide nitrogen donor atoms.[393a] The stability of the complexes $Cu(L\text{-}4H)^{2-}$ decreases somewhat with increasing *n* in structure **67** (from $n = 2$ to $n = 4$).[393a] Co^{3+} and Cr^{3+} complexes of similar ligands, derived from azo dyes, have been isolated and characterized;[393b] these complexes also contain ionized sulfonamide moieties.

Sulfonamides are also effective inhibitors of the Zn^{2+}-containing enzyme carbonic anhydrase. Typically the sulfonamide group of the free inhibitor deprotonates with $pK_a = 7$–10. This range of values is less than the $pK_a = 12$ for *N*-tosyl-α-alaninate referred to above. Though there has been controversy, the sulfonamide inhibitors appear to bind as anions through the deprotonated sulfonamide nitrogen to the Zn^{2+} in carbonic anhydrase.[394] This binding is strengthened considerably by hydrophobic and related interactions between the aromatic moiety of the sulfonamide and nonpolar groups of the active site cavity. Hydrophobic ligand–ligand interactions are also known to enhance the stability of small mixed-ligand complexes.[170,173]

XVII. Other Ligating Groups Related to the Amide Function

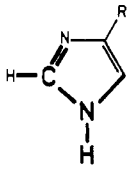
The structural unit **68**



68

is not only present in the amide group, but also in a number of other functions. To emphasize this similarity the above structural unit is drawn with *heavy lines* in all structures shown in this section.

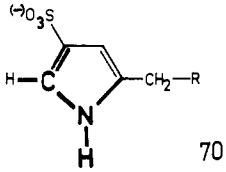
The structural unit **68** is part of heteroaromatic ring systems and occurs, e.g., in imidazole (**69**) and pyrrole



69

residues. The nitrogen-bound hydrogen of imidazole is released with pK_a = 14.2,[52] and this hydrogen may be substituted by metal ions as already discussed in section XID. This substitution occurs with formation of binuclear (or polynuclear) complexes; i.e., the imidazolate system may bridge two metal ions. Such an imidazolate bridge was also found in superoxide dismutase between Cu^{2+} and Zn^{2+}.[395,396]

The ionization of the nitrogen in pyrrole occurs with pK_a = 17.5[397] and that of the pyrrole nitrogen in 2-[(methylamino)methyl]pyrrole-4-sulfonate (**70a**) with

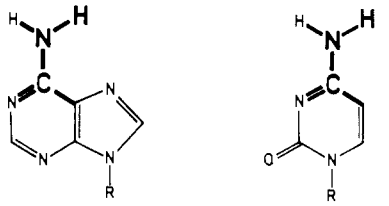


70

70a: R = $-NH-CH_3$
b: = $-NH-CH_2-CH_2-NH_2$
c: = $-NH-CH_2-COO^-$
d: = $-N-(CH_2-COO^-)_2$

pK_a = 15.4;[398] hence, the basicity of the pyrrole nitrogen is comparable with that of the amide nitrogen (sections IIA and IIC). Substitution of the hydrogen by a metal ion is therefore again only possible if strong primary binding sites are available. The pyrrole ligand **70a** is not able to prevent metal ion hydroxide precipitation with Ni^{2+} and Cu^{2+}, whereas ligand **70b** with the bidentate ethylenediamine residue is able to do so.[398] The corresponding complexes $M(L)^+$ with Cu^{2+} and Ni^{2+} are deprotonated with pK_a = 6.5 and 9.2, respectively, to give M(L-H); the difference of about 2.7 log units between the acidifying power of these two metal ions corresponds to the observations made with peptides (Table I). With ligands **70c** and **70d** only Cu^{2+} forms $Cu(L-H)^{-}$ or $^{2-}$ complexes; the proton is released with pK_a = 6.8 and 8.0, respectively.[398] Those ligands which form M(L-H) complexes allow in addition the formation of the species M(L)(L-H) and M(L-2H).[398] Some other pyrrole ligands have also been studied.[399]

Another class of ligands that contains the structural unit **68** is derived from amino-substituted aromatic moieties. Such amino groups are very poor electron donors and therefore also poor binding sites; this is reflected in the coordinating properties of purine and pyrimidine nucleotides: in these ligands the amino group does *not* participate in complex formation.[400] Deprotonation of such an amino group, which should transfer it into an excellent ligating group, is again not easily achieved; it occurs in adenosine (**71**) with pK_a =

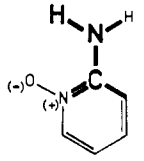


R = β-D-ribofuranosyl

71 72

16.7.[401] Hence, it is not surprising that the first examples of adenosinate and cytidinate (cytidine = **72**) complexes have been described only recently. The tripositive pentaammineruthenium(III) complex facilitates this deprotonation and coordinates at the ionized exocyclic amine nitrogen.[402,403] In basic or slightly acidic solutions methylmercury (CH_3-Hg^+) may also substitute for an amino group proton of 9-methyladenine, which already has a methylmercury bound to N-1.[404]

N^1-Oxide formation of adenosine (**71**) facilitates complex formation considerably because a primary binding site allowing chelate formation is introduced into the molecule, and the amino group is remarkably acidified (pK_a = 12.86).[405] Indeed the deprotonated 6-amino N^1-oxide group of adenosine derivatives is an effective chelator not only for Cu^{2+} but also for Mn^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+}.[405-407] Similarly, 2-aminopyridine 1-oxide (**73**) forms only very weak complexes in its



73

neutral form[408] while it complexes well with Cu^{2+} and Fe^{3+} as an anion that has lost a proton from the 2-amino group.[409]

XVIII. Summary of Metal Ions That Substitute for an Amide Hydrogen

As described in section II, metal ions may interact weakly with the carbonyl oxygen of a neutral amide group. This interaction becomes more favored in the presence of an anchoring group capable of forming a chelate ring with a metal ion and the carbonyl oxygen, but even with chelation, this interaction remains weak.

Substitution of a nitrogen-bound amide hydrogen by a metal ion provides a much stronger coordination than does the carbonyl oxygen of the neutral amide group.

As indicated in Figure 1, the deprotonated amide nitrogen is about 10^{16} times more basic than the neutral amide group. Due to the high basicity of the deprotonated amide nitrogen with $pK_a \simeq 15$, metal ion hydrolysis often competes with metal ion substitution of an amide hydrogen. The balance shifts to metal ion substitution at an amide group when one or more anchors occur so that favorable 5- or 6-membered chelate rings may form. A major portion of this review summarizes research that describes effective anchors for chelate ring formation containing deprotonated amide nitrogens. Among the groups providing effective anchors in proteins are other amide groups in the polypeptide backbone, the imidazole side chain of histidine residues, and the sulfhydryl side chain of cysteine residues.

As reviewed in section IV, the order of peptide hydrogen displacement by metal ions with a representative pK_a value in parentheses for short peptides is given by Pd^{2+} (2) > Cu^{2+} (4) > Ni^{2+} (8) > Co^{2+} (10). Many investigations have been conducted with Cu^{2+} and Ni^{2+}; examples occur in almost every section of this article. The corresponding trivalent ions represent a special topic, covered in section VIII. A lesser number of investigations have involved planar Pd^{2+} (sections IIIB, IVC, IVF, VC, VIB, VIIB, IX, X, XIA, XII, and XVI) and Pt^{2+} (sections IVF, VA2, and XVIA) complexes. Many Co^{2+} complexes (sections IIIB, IIIC, IVD, IVE, IVF, VA2, XIA, XIC, XIIIA, XIV, XV, XVI, and XVII) undergo oxygenation and oxidation to Co^{3+} species (sections IVD, IVF, VIIA, XIE, XIVA, XVIA2, and XVIB). For Zn^{2+}, metal ion hydrolysis often competes with substitution at an amide nitrogen, but bona fide examples of the latter reaction seem to exist (e.g., sections IVE, XIA, XII, XIIIA, XVIA, and XVII). Since substitution of a peptide hydrogen by Zn^{2+} occurs just within the physiological pH region, if such a complex does occur in proteins, facile exchange may render it difficult to retain and detect.

From the absence of reported results in the literature it is evident that alkali, alkaline earth, and first row transition-metal ions other than those mentioned fail to substitute for a peptide hydrogen in the typical model systems studied. Undoubtedly there have been numerous unsuccessful and thus unreported attempts to detect such an interaction.

Deprotonated peptide nitrogen–metal ion interactions seldom occur in biological systems. A deprotonated peptide nitrogen–Cu^{2+} bond may occur naturally with small peptides such as carnosine (section XID). Cu^{2+} interaction at the amino terminus of albumin might play some role in Cu^{2+} transport (section XV);[358] conceivably this role has been or will become more important in the course of evolution. Claims for peptide nitrogen deprotonation in several metalloproteins are unjustified (section XV).

The biosynthesis of bleomycin (section XIIIA) provides an important example of the biological use of deprotonated peptide nitrogen–Cu^{2+} interactions. The low tendency for natural systems to incorporate deprotonated peptide–Cu^{2+} multidentate chelates may be due to the resulting slow exchangeability of Cu^{2+} and incompatibility of the chelate with secondary structures such as the α helix and with a reduced Cu^{+} state. The main role of a multidentate deprotonated peptide chelate appears to be in stabilizing groups in a fixed geometry. Perhaps this is the function of Cu^{2+} in bleomycin biosynthesis. Other metal ions including Fe^{2+} may also deprotonate an amide nitrogen in bleomycin (section XIIIA). This is the only validated instance where Fe^{2+} initiates an amide deprotonation, and demonstrates again that biological molecules may provide unusual ligands.

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