Visible Circular Dichroism of Copper(II) Complexes of Amino Acids and Peptides[1]

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Abstract: Most copper(II) complexes of L-amino acids and peptides exhibit a negative Cotton effect throughout the predominant band in the visible spectrum from 620 to 490 nm. L-Proline complexes yield a net positive circular dichroism (CD). The magnitude of CD in copper(II)–dipeptide complexes Cu(X-X) may be accurately estimated by adding the magnitudes of the corresponding glycyl dipeptide complexes, Cu(gly-X) and Cu(X-gly), indicating that the CD is an additive function of independent contributions from amino and carboxyl terminal amino acid residues. Addition of the negative CD magnitudes for the copper(II) complexes of the three tripeptides composed of one L-leucyl and two glycyl residues yields the value obtained experimentally for the complex of the tripeptide composed of three L-leucyl residues. These results cannot be accounted for by any octant or quadrant rule. A hexadecant rule accounts for the sign identity and magnitude additivity observed in these complexes. To form hexadecants, the coordination plane about copper(II) ion is divided perpendicularly into eight wedge-shaped sectors of alternating sign centering on the metal ion. The eight sectors above the plane possess signs opposite those of the eight sectors below the plane. In the case of a copper(II)–tripeptide complex, all L-amino acid side chains appear in sectors of identical sign so that sign identity and magnitude additivity are accommodated. The hexadecant rule appears applicable to the ligand-field bands of tetragonal transition metal ion complexes where vicinal effects of substituents generate the optical activity.

Extensive titration, spectrophotometric,[2] and X-ray diffraction[3] studies have led to a clear understanding of the structures of copper(II) complexes of amino acids and simple peptides. Nearly planar chelate rings are formed in copper(II) complexes of α-amino acids.[3] Copper(II) promotes the ionization of amide hydrogens in neutral solutions of simple peptides, yielding chelates containing planar, *trans* amide bonds with trigonal amide nitrogens as donor atoms.[2] These tetragonal copper(II)–peptide complexes provide a relatively unpuckered system of chelate rings to which side chains are attached in known dispositions.[3] An excellent series of compounds is therefore available for investigation of the optical rotatory properties of copper(II) complexes. In a preliminary communication the inapplicability of any octant rules to peptide complexes has been shown.[4] An alternative sector rule that better describes the results was suggested and its applicability is discussed in this paper.

The blue copper(II) complexes of amino acids and peptides exhibit weak absorption in the visible region due to transitions that take place within predominantly d orbitals on the metal ion. In this paper we report the circular dichroism (CD) results obtained in the d–d transitions of a variety of copper(II) complexes of optically active amino acids and peptides. Results on histidine and other tridentate complexes of copper(II) are reported separately.[5] The effects of chelation by copper(II) on ligand bands and electron transfer bands in the ultraviolet have been presented.[6] Application of the results reported here to the binding of copper(II) to some proteins has been made.[7]

Experimental Section

N-Methyl-L-proline·HCl was prepared from L-proline and formaldehyde in formic acid,[8] and the decomposition point agreed with a literature value.[9] All other ligands were high-quality commercial products. Circular dichroism was measured on a Durrum-Jasco ORD/UV-5 recording spectropolarimeter with a CD attachment. The long wavelength limit for CD measurements was about 700 nm. All CD magnitudes are reported as the difference in molar absorptivity, based on copper(II) concentration, between left and right circularly polarized light. These differential molar absorptivities are designated as $\Delta\epsilon$. Other details have been supplied.[10] All experiments were performed at room temperature, near 23°.

Results

A typical absorption spectrum of 2:1 complexes of amino acids with copper(II), prepared with the addition of sufficient base so that the α-amino group is basic amine, exhibits a maximum at about 620 nm with a molar absorptivity $\epsilon \sim 50$. A typical CD spectrum of the same solutions exhibits two extrema, which for L-amino acid complexes are negative in the 590–620-nm region and positive at 700 nm, the long wavelength limit of the instrument available in this study. Other workers have shown a small positive $\Delta\epsilon \sim +0.05$ at about 730 nm.[11] The greatest magnitude CD in 2:1 L-amino acid–copper(II) complexes appears in the region of the absorption maximum, is normally negative, and occurs in the 590–620-nm region with an occasional shoulder also of negative sign on the CD curve at lower wavelengths, such as 575 nm in the case of alanine. We label the positive CD near 700 nm

(1) This paper is abstracted from the Ph.D. thesis (1967) of John M. Tsangaris and the research was supported by a grant from the National Science Foundation.

(2) For a summary, see W. L. Koltun, R. H. Roth, and F. R. N. Gurd, *J. Biol. Chem.*, 238, 124 (1963).

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(4) R. B. Martin, J. M. Tsangaris, and J. W. Chang, *J. Amer. Chem. Soc.*, 90, 821 (1968). Hexadecant seems a more appropriate name than the double octant employed earlier.

(5) E. W. Wilson, Jr., M. H. Kasperian, and R. B. Martin, *ibid.*, in press.

(6) J. M. Tsangaris, J. W. Chang, and R. B. Martin, *ibid.*, 91, 726 (1969).

(7) J. M. Tsangaris, J. W. Chang, and R. B. Martin, *Arch. Biochem. Biophys.*, 103, 53 (1969).

(8) H. T. Clark, H. B. Gillespie, and S. Z. Weisshaus, *J. Amer. Chem. Soc.*, 55, 4571 (1933).

(9) H. N. Christensen and R. R. Riggs, *J. Biol. Chem.*, 220, 265 (1956).

(10) J. W. Chang and R. B. Martin, *J. Phys. Chem.*, 73, 4277 (1969).

(11) T. Yasui, *Bull. Chem. Soc. Jap.*, 38, 1746 (1965); T. Yasui, J. Hidaka, and Y. Shimura, *J. Amer. Chem. Soc.*, 87, 2762 (1965).

as band I, and the two bands occurring at successively shorter wavelengths as bands II and III, respectively. Magnitudes of CD at extrema shown in the second column of Table I for 2:1 L-amino acid–copper(II)

Table I. Circular Dichroism Extrema from 590 to 670 nm in Copper(II) Complexes of L-Amino Acids (2:1) and Glycyl Dipeptides (1:1)

L-Amino acid, X	2X	Gly-X	X-Gly
Aspartic acid	−0.03	−0.26	+0.03
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

ion complexes refer to the most negative portion of bands II and III. The results agree with recorded spectra for some of these complexes.[11] Values of $\Delta\epsilon$ for 2:1 complexes of L-amino acids not listed in Table I are glutamine, −0.13; lysine, −0.16; and phenylglycine, −0.50. In these complexes the carboxylic acid side chains of aspartic and glutamic acids are in their anionic carboxylate forms, and the side chains of lysine and arginine bear a positive charge, while the side chains for all other ligands are uncharged so that the net charge on these last complexes is zero.

All the 2:1 L-amino acid–copper(II) complexes mentioned so far exhibit positive CD in band I near 700 nm and a larger negative CD in band II at 590–620 nm. Exceptions are the imino acids, proline, where $\Delta\epsilon = +0.33$ and -0.07 at 665 and 525 nm, respectively, and hydroxyproline, where $\Delta\epsilon = +0.35$ at 645 nm. These results are in agreement with recorded spectra.[11,12] Thus both imino acid complexes exhibit a positive CD for band II which is greater than the negative CD of band III. On the other hand, N-methyl-L-proline forms a 2:1 copper(II) complex that gives $\Delta\epsilon = -0.18$ at 620 nm similar to that of a typical 2:1 amino acid–cupric ion complex listed in the second column of Table I. The absorption spectra of the proline and hydroxyproline complexes give maxima at 612 nm with $\epsilon = 65$, while for the N-methylproline complex the maximum at 620 nm yields a relatively high $\epsilon = 156$. As has been pointed out,[11] formation of a chelate ring in proline in addition to the pyrrolidine ring is possible with only one configuration of the tetrahedral nitrogen so that, in effect, a second asymmetric center is produced in the ligand as a result of chelate formation. The magnitude of this asymmetry is reduced upon methylation to give N-methyl-L-proline, for now two nitrogen substituents are hydrocarbon residues. The relatively low $\Delta\epsilon$ observed for this complex demonstrates that a second asymmetric center within a chelate ring need not necessarily produce a large circular dichroism.

(12) K. M. Wellman, S. Bogdansky, C. Piontek, C. R. Hare, and M. Mathieson, *Inorg. Chem.*, 8, 1025 (1969). By analyzing the CD curves of prolines into two instead of three bands these authors made mistaken comments on our preliminary communication.[4] In any case one electron perturbation model sector rules apply to the net rotatory strength over all d–d transitions: F. S. Richardson, private communication.

In contrast to the change in sign for band II on comparing the copper(II) complexes of proline and alanine, the 2:1 complexes of L-prolinamide and L-alaninamide, prepared at about pH 11 so that the amide hydrogens are ionized, both exhibit negative CD in the visible region. The L-alaninamide complex yields $\Delta\epsilon = -0.25$ at 548 nm, and the L-prolinamide complex yields a double peaked curve with $\Delta\epsilon = -0.27$ and -0.36 at 560 and 472 nm, respectively. Both 2:1 amide–copper(II) complexes exhibit absorption maxima with $\epsilon \sim 65$ near 525 nm reflecting four nitrogen donors. The CD of the copper(II) complex Cu–(1,2-diaminopropane)$_2$ yields $\Delta\epsilon = +0.27$ at 510 nm.[13] Since the ligand used may be correlated with D-amino acids, the sign and magnitude are identical with those of a D-alaninamide complex even though puckering in the chelate rings should be much less for alaninamide.

Owing to the carboxylate side chain, the 2:1 aspartic acid–cupric ion complex may be 5- or perhaps even 6-coordinated. In addition to the weak CD listed in the second column of Table I, a small $\Delta\epsilon = +0.01$ appears at 560 nm. The positive value for band III might indicate additional chelation above or below the plane. A positive value is also observed for band III in the 2:1 L-threonine complex in the presence of two additional equivalents of base where $\Delta\epsilon = +0.03$ at 470 nm. We suggest that this positive value for the short wavelength band might indicate binding of the threonine alcoholate groups above or below the plane. The absence of such a positive CD peak in the serine complex suggests either that this alcoholate group is bound more weakly or that dissymmetric groups such as the second asymmetric center in threonine or a carboxylate group as in aspartic acid are required for a positive peak to appear. The 2:1 complexes of both serine and threonine show an apparent displacement of bands I and II to shorter wavelength CD on addition of excess base with an increase in magnitude of band I and a decrease in the magnitude of band II. A reduction in CD magnitude also occurs in band II of proline and hydroxyproline complexes on addition of two additional equivalents of base. Apically coordinated water may have ionized in these two cases; the hydroxy group hydroxyproline is not in a position to coordinate. Other 2:1 complexes of amino acids usually gave precipitates on addition of excess base. Addition of up to 24 times as much anionic amino acid ligand as copper(II) gives no qualitative change in visible CD or absorption spectra compared to the 2:1 complexes in the cases of alanine, valine, serine, and threonine.

Solutions containing amino acids and cupric ions in a 1:1 molar ratio also exhibit a negative CD but displaced about 50 nm to longer wavelengths and with about one-third the magnitude of the corresponding 2:1 complexes. For the same bands the CD signs of 1:1 complexes seem to correspond in all cases to those of the more instrumentally accessible 2:1 complexes.

The $\Delta\epsilon$ values for the CD extremum of band II near 650 nm of 1:1 complexes of dipeptides composed of glycine and one other amino acid under pH conditions where the amide hydrogen is ionized are shown in Table I. An absorption maximum appears near 636 nm with $\epsilon \sim 80$ for these complexes with one amino nitrogen, one amide nitrogen, and one carboxylate

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

oxygen donor atoms from each ligand with water occupying the fourth coordination position. Ignoring any change on the amino acid side chains the complexes are of the form CuL^0. Often a shoulder is observed in the CD curve at about 540 nm (band III) with the same sign as the peak at longer wavelength. The third column of Table I which lists the CD magnitudes for glycyl-L-amino acid dipeptides exhibits greater magnitudes than the fourth column where values for L-aminoacylglycines are recorded. The L-amino acid residues in Table I are arranged in approximate order of increasing negative value in the third column. There is some correlation of this order with increasing negative values for the amino acid chelates, with increasing size of the side chain, and with the transition from negative to positive values in the last column of Table I. Evidently the change of sign in the last column does not represent a sharp transition in some property of the complex, but results from gradually increasing size in the side chains.

In Table II are listed the observed circular dichroism extrema for band II near 650 nm for 1:1 copper(II) complexes of dipeptides with ionized amide hydrogens (CuL^0) composed of L-amino acids and the values calculated for the same band from Table I by adding the observed $\Delta\epsilon$ values for glycyl-containing dipeptides. For example, the calculated value for the third entry in Table II, L-Ala-L-Leu, is obtained by adding the values for L-alanylglycine and glycyl-L-leucine in Table I. The calculated value for L-Ala-D-Ala in Table II is obtained by subtracting the value of glycyl-L-alanine in Table I from the value for L-alanylglycine in the same table. The agreement between the observed and calculated values in Table II extends to cases where positive values for the X-Gly contribution are included in the calculation for D-amino acid residues or aromatic side chains. The good agreement demonstrates that the circular dichroism in 1:1 copper(II)–dipeptide complexes is an additive function of independent contributions from amino and carboxyl terminal amino acid residues.

Table II. Circular Dichroism Extrema near 650 nm of Copper(II) Complexes of Dipeptides Composed of L-Amino Acid Residues

Dipeptide	Observed	Calcd from Table I
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

Upon addition of 2 additional equiv of base, so that the water in the fourth coordination position of the 1:1 complexes is ionized to give $CuLOH^-$, Gly-Val, Gly-Ser, and Gly-Thr complexes all exhibit a similar apparent separation of the two negative bands II and III, from about 660 and 540 nm in the complexes of Table I to 680 and 510 nm with excess base with a tendency toward more equal magnitudes in the presence of excess base. No positive component as in the amino acid threonine–copper(II) complex was detected in any case. The similarity in behavior of the three dipeptides upon addition of sufficient excess base to ionize the hydroxy side chains indicates that if apical chelation is occurring, it is not easily detectable by circular dichroism. Compared to the free amino acid complexes, the ionized planar trigonal amide group provides a conformational restraint not present in the free amino acids, and apical chelation is less probable in these dipeptides. However, in the presence of excess base a 1:1 mixture of glycyl-L-asparagine and copper(II) yields three negative CD bands. The accompanying shift in the absorption maximum to 574 nm suggests a complex of unusual structure with three nitrogen donor atoms.

Though shifted to shorter wavelengths by about 30 nm in the absorption maximum and about 90 nm in CD, 1:1 complexes of dipeptide amides with three nitrogen donor atoms (CuL^0) exhibit magnitudes similar to those of the corresponding dipeptides. As with the dipeptide complexes, addition of another equivalent of base results in ionization of coordinated water to yield $CuLOH^-$ and bands II and III separate into distinct CD peaks in this case at about 600 and 490 nm, as shown in Table III. The absorption maxima appear at about 570 nm with $\epsilon \sim 75$. The last two entries in Table III indicate that bulky substituents in an amino terminal position may yield a positive sign for the CD of band II. Results for the neutral complexes of dipeptide amides are not presented because titration curves, the observed blue shift from about 605 to 570 nm when a small red shift is expected for water replacement by hydroxide ion, and the magnitudes of the CD curves all indicate that the solutions contain mixtures of CuL^0, $HLCuOH^0$, and perhaps other complexes as well.

Table III. Circular Dichroism of Copper(II) Complexes ($CuLOH^-$) of Dipeptide Amides Composed of L-Amino Acids

Dipeptide amide	~600 nm	~490 nm
Gly-Ala-NH_2	−0.49	−0.29
Gly-Leu-NH_2	−0.65	−0.30
Gly-Phe-NH_2	−0.75	−0.35
Phe-Phe-NH_2	−0.76	−0.35
Val-Gly-NH_2	+0.04	−0.05
Tyr-Gly-NH_2	+0.18	−0.02

Most of the 1:1 copper(II) complexes of tripeptides listed in Table IV yield only a single CD extremum at about 560 nm for bands II and III. Both peptide nitrogens have undergone deprotonation and are bound to copper(II) in these complexes so that the complexes possess a net negative charge (CuL^-). Two tripeptide complexes with bulky amino terminal side chains listed in Table IV exhibit single positive extrema. Absorption maxima for the copper(II)–tripeptide complexes occur near 550 nm with molar absorptivities of about 150. Though the peak is shifted to a lower wavelength, two tripeptide amides recorded in Table IV yield CD magnitudes similar to those of the corresponding tripeptides. This similarity indicates that substitution of N for O donors does not significantly alter CD signs or magnitudes of tripeptides and suggests that D_{4h} microsymmetry is a satisfactory approximation for tripeptides. Lysine vasopressin, the last entry in Table IV, is a partially cyclic nonapeptide composed of L-amino

Table IV. Circular Dichroism Extrema of Copper(II) Complexes of Tripeptides Composed of L-Amino Acid Residues

Tripeptide	$\Delta\epsilon$ at ~560 nm
Leu-Gly-Gly	−0.20
Gly-Leu-Gly	−0.89
Gly-Gly-Leu	−0.76
Sum	−1.85
Leu-Leu-Leu	−1.92
Gly-Gly-Leu-NH_2	−0.85[a]
Gly-Ala-Gly	−0.68
Gly-Ala-Leu	−1.27
Val-Gly-Gly	−0.08
Pro-Gly-Gly	+0.30
Phe-Gly-Gly	+0.94
Gly-Phe-Gly	−0.60
Gly-Gly-Phe	−0.96
Phe-Phe-Phe	−1.9
Gly-Gly-Phe-NH_2	−0.91[a]
Lysine vasopressin	−1.25[a]

[a] Extrema at 530 nm for these tripeptide amides and lysine vasopressin.

acids with an N terminal half-cystine residue followed successively by tyrosine, phenylalanine, and glutamine residues. The absorption maxima at 530 nm observed in the presence of an equivalent of copper(II) after ionization of three amide hydrogens strongly suggests copper(II) attachment at the amino terminus and the three succeeding peptide nitrogens.[14]

Absorption maxima and CD extrema appear at about 15 nm shorter wavelength for copper(II) complexes of tripeptide amides than for the 2:1 complexes of amino acid amides, a result indicating that the ligand field strength of ionized amide nitrogen donors is greater than that for amino donors. The identical ordering is observed for the corresponding nickel(II) complexes.[10] The reverse ordering has been suggested on the basis of a single comparison of copper(II) complexes of a pair of peptide ligands.[15] The ordering may depend upon the substituents on the nitrogen atoms, and it may be concluded that the two ligand-field strengths are comparable. This conclusion is consistent with electron spin resonance experiments on peptide complexes of copper(II) where both kinds of nitrogen atoms appear magnetically equivalent.[16]

Discussion

In the majority of cases the transition designated as band II exhibits a dominant negative CD sign for the copper(II) complexes of L-amino acids and peptides. The position of this band moves to shorter wavelengths as the number of nitrogen donors increases. Complexes of the imino acids L-proline and L-hydroxyproline yield positive $\Delta\epsilon$ values for band II but L-prolinamide and L-N-methylproline complexes give negative signs. Bulky side chains in the amino terminal positions of dipeptide, dipeptide amide, and tripeptide copper(II) complexes yield positive Cotton effects in the region of band II. Until the difference in sign between the proline and prolinamide complexes is understood, it seems premature to apply a simple sector rule to this cyclic system.

One of the most interesting results of this research is the identical sign for L-amino acid residues and additivity of mangitudes in the CD of copper(II) complexes of peptides. Except for those dipeptides with bulky side chains in amino terminal positions, the complexes in Table I exhibit negative signs for band II. Furthermore as Table II demonstrates, the magnitude of the CD for all the dipeptide complexes studied can be accounted for by adding the magnitudes of the constituent glycyl-containing dipeptide complexes.

The major points of CD sign identity and magnitude additivity are elaborated by the results recorded for the first four entries in Table IV. Three tripeptide complexes composed of two glycyl and one L-leucyl residue in three positions all exhibit negative CD, and furthermore, the sum of their $\Delta\epsilon$ values nearly equals the $\Delta\epsilon$ value of the copper(II) complex of the tripeptide containing three L-leucyl residues. A similar CD sign identity and magnitude additivity has been observed in copper(II),[17] nickel(II),[10,17] and palladium(II)[18] complexes of tripeptides composed of L-alanyl residues. Another example is found in Table IV, as the sum of the CD for the copper(II) complexes of Gly-Gly-Leu and Gly-Ala-Gly is only slightly greater than the observed value for the Gly-Ala-Leu complex. On the other hand neither sign identity nor magnitude additivity is observed in the set of phenylalanyl tripeptide complexes tabulated at the bottom of Table IV.

The sign identity and additivity of CD magnitudes demonstrated in Tables I and II and at the top of Table IV cannot be accommodated by any octant rule for sign accounting in tetragonal transition metal ion complexes. No matter how octants are drawn, octant rules predict that the sign observed for Gly-L-Leu and L-Leu-Gly complexes, for instance, should be opposite, and that the sign for the Gly-L-Leu-Gly complex should be opposite to that of the other two tripeptide complexes containing one L-leucyl residue. Proposed octant rules for d–d transitions in metal ions are based on a knowledge of chelate ring puckering.[19] The copper(II) chelates of amino acids and peptides reported in this research are nearly planar and less puckered than the ethylenediamine type chelates often studied. The main deviation from planarity in solid copper(II) complexes of amino acids and peptides appears to be small displacements of copper(II) from the plane of the four donor atoms in the direction of a group coordinated in an apical position.[3] In solution there is apt to be a more averaged position for the metal ion with a rapid exchange of solvent groups above and below the plane of the chelate ring inhibited to some extent on the side carrying the amino acid side chain.

Sign identity and magnitude additivity observed in the CD of tetragonal tripeptide complexes may be accommodated by a hexadecant rule.[4] Hexadecants are illustrated in Figure 1 where the plane of the metal ion complex is divided perpendicularly into eight wedge-shaped sectors centering on the metal ion. There are

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(15) G. F. Bryce and F. N. R. Gurd, *J. Biol. Chem.*, 241, 122 (1966).
(16) D. C. Gould and H. S. Mason, *Biochemistry*, 6, 801 (1967); G. F. Bryce, *J. Phys. Chem.*, 70, 3589 (1966); K. E. Falk, H. C. Freeman, T. Jansson, B. G. Malmstrom, and T. Vanngard, *J. Amer. Chem. Soc.*, 89, 6071 (1967).
(17) G. F. Bryce and F. R. N. Gurd, *J. Biol. Chem.*, 241, 1439 (1966).
(18) E. W. Wilson, Jr., and R. B. Martin, *Inorg. Chem.*, 9, 528 (1970).
(19) C. J. Hawkins and E. Larsen, *Acta Chem. Scand.*, 19, 185 (1965); K. M. Wellman, W. Mungall, T. G. Mecca, and C. R. Hare, *Chem. Eng. News*, 45, 48 (Oct 2, 1967).

eight sectors of alternating optical rotatory sign above the plane of the chelate ring and eight below, making a total of sixteen sectors. All adjacent sectors are assigned opposite signs so that the sign changes on passing perpendicularly through the chelate plane. The dark and light areas in Figure 1 represent signs such that dark areas might be assigned positive signs and all light areas negative signs.

The hexadecant rule depicted in Figure 1 can account for CD sign identity and magnitude additivity in copper(II) complexes. The metal ion and atoms of the chelate rings are in a nodal plane so that they do not contribute to optical activity. Each of the L-amino acid side chains on a di- or tripeptide lies in a sector of the same sign assignment on one side of the chelate plane. The relatively rigid planar conformation of the peptide group with a trigonal nitrogen that has undergone amide hydrogen ionization restricts side chains in nonamino-terminal amino acid residues to one sector. *trans*-Bidentate chelates in tetragonal complexes would in general obey either octant or hexadecant rules. It is the study of substituents on adjacent quarters of a square chelate afforded by di- and tripeptides that provides the critical test for the superiority of the hexadecant rule for planar or near-planar chelates. The hexadecant rule has also been found to be applicable to tetragonal nickel(II)[10] and palladium(II)[18] complexes.

The hexadecant Figure 1 is a representation of the pseudoscalar function for the D_{4h} point group which gives the regional rule in the one-electron static asymmetric perturbation model of optical activity.[20] The frequency of nodal planes is evidently not too great to prevent its application to relatively rigid tetragonal peptide metal ion complexes. Pseudoscalar representations[20] for D_{2h} (octant) or C_{2v} (quadrant) point groups give regional rules which do not account for sign identity and magnitude additivity observed in the di- and tripeptide complexes.

The pseudoscalar representation for the C_{2h} or C_s point groups simply assigns one sign above and the opposite sign below the plane of the chelate ring.[20] The C_{2h} rule predicts that the CD magnitudes would be additive in *trans*- and subtractive in *cis*-tetragonal complexes of bidentate chelates such as L-amino acids. Thus *trans* complexes of L-amino acids or derivatives would exhibit appreciably greater optical activity than *cis* if the C_{2h} rule were valid. The C_{2h} rule may be eliminated by the following comparison. Side chains are on opposite sides of the chelate planes in the necessarily *cis*-copper(II) complex of N,N′-bis-L-alanylethane-1,2-diamine where $\Delta\epsilon = -0.65$.[21] Addition of the $\Delta\epsilon$ results[17] for the copper(II) complexes of L-alanylglycylglycine (-0.19) and glycylglycyl-L-alanine (-0.48) predicts $\Delta\epsilon = -0.67$ for L-alanylglycyl-L-alanine, a *trans* complex with both side chains on the same side of the chelate plane. The nearly identical magnitudes for these *cis* and *trans* complexes with methyl side chains rule out a C_{2h} rule and is consistent with the hexadecant (D_{4h}) rule. Nearly identical CD magnitudes in relatively inert *cis*- and *trans*-palladium(II) complexes of L-alaninamide also eliminate the C_{2h} rule for tetragonal complexes.[18,22] Alternatively, a C_{2h} or octant rule may be applicable to *trans* and a C_{2v} rule to *cis* complexes. If so the generality afforded by a single rule is lost.

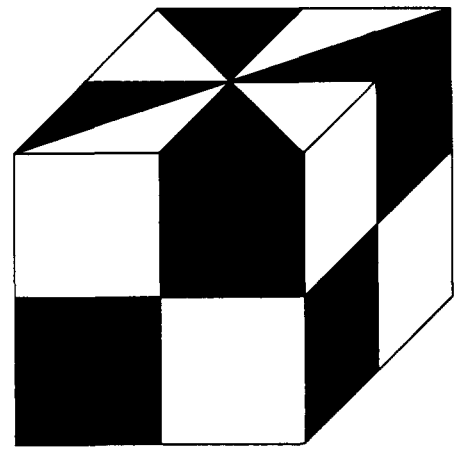

Figure 1. Representation of the hexadecant rule for tetragonal complexes. Metal ion is at the center and donor atoms are at the vertical edges of the cube, where there is a color change, but the dark and light regions extend indefinitely. A two-dimensional representation also showing locations of ligand atoms appears in ref 4 and 10.

Provided that large side chains do not occur in amino terminal residues of peptides, the hexadecant rule appears to be an appropriate description of the optical activity in tetragonal metal ion complexes of amino acids and peptides. Rather than the usual negative signs for L-amino acid residues, positive signs occur in Tables I, III, and IV when bulky side chains appear in the amino terminal residues of peptides. A study of dipeptide maps reveals that side chains in other than amino-terminal residues must be at least quasi-axial in tetragonal metal ion complexes. Steric hindrance with the amide oxygen atom of the preceding residue disallows an equatorial disposition for side chains in nonamino-terminal residues.[23] Unlike the rigid geometry about an ionized trigonal peptide nitrogen atom, a tetrahedral nitrogen atom in an amino-terminal residue is less restricted, and a bulky side chain may take up a position equatorial to the chelate plane. Large side chains in an equatorial position may adopt average positions in adjacent sectors and displace the α-carbon from the chelate nodal plane into a sector of signs opposite to that of a smaller side chain. The tendency for occurrence of an equatorial conformation even with smaller side chains may account for the lower CD magnitudes of the same side chain in an N-terminal compared to some other position in a peptide. Evidence supporting the suggestion of the importance of equatorial conformers in amino-terminal residues with bulky side chains is found in the copper(II) complexes of ligands like L-2-amino-1-propanol which exhibit a net positive CD.[24] In the 2-amino-1-propanol complex the chelate ring is puckered and an equatorial position for the side chain should be favored. The side chains of amino acid residues chelated through planar trigonal amide groups are not permitted the conformational

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adaptability of their amino-terminal counterparts, with the result that positive CD does not occur for L-amino acid residues in nonamino-terminal positions.

The hexadecant rule represents a description of optical activity in the ligand-field bands of tetragonal transition metal ion complexes due to vicinal effects of substituents. For the other than amino-terminal residues vicinal effects represent the primary contribution to the relatively weak optical activity in peptide complexes. When conformational contributions become significant or generation of optical activity occurs through a coupled dipole mechanism additional factors enter the picture.

It does not seem possible at this time to assign unequivocally the experimentally observed bands I, II, and III to the d–d transitions taking place predominantly on copper(II) ion. The designations of the parent D_{4h} symmetry point group are utilized because this group represents the microsymmetry of the donor atoms about the metal ion as it has been shown that substitution of oxygen for nitorgen makes no significant difference. It now seems generally agreed that, for nitrogen and oxygen donor systems like those considered here, the order of increasing energy for 3d orbitals in copper(II) complexes is $yz \sim xz < xy < z^2 < x^2 - y^2$, where the x and y coordinates in the plane of the copper(II) complex point toward the donor atoms in the coordination plane.[25] For the D_{4h} symmetry group the above order of increasing energy of copper(II) 3d orbitals is $e_g < b_{2g} < a_{1g} < b_{1g}$. In the d^9 copper(II) system the three one-electron d–d transitions in order of increasing energy are $B_{1g} \rightarrow A_{1g} < B_{1g} \rightarrow B_{2g} < B_{1g} \rightarrow E_g$. For brevity we shall designate these transitions as A, B, and E, respectively. In the D_{4h} symmetry group the first of these transitions, A, is magnetic dipole forbidden, while the last two are magnetic dipole allowed. Therefore, the lowest energy transition, A, is expected to give rise to weak CD or none, the nearer D_{4h} microsymmetry is approached by the donor atoms. In complexes that deviate appreciably from D_{4h} symmetry, the degenerate e_g orbitals may be split so that transition E will consist of two components.

The simplest course is to identify bands I, II, and III with transitions A, B, and E, respectively. Two lines of evidence indicate that there is one band in addition to band I at wavelengths longer than 700 nm. A positive shoulder is observed near 820 nm in the CD of L-amino acid complexes[11] and a peak at 870 nm is reported in the absorption spectra of copper(II) complexes of dipeptides.[26] We have performed both of these measurements and are unable to find any significant absorption at all at 870 nm in the latter case. Careful examination of the CD curves produced by amino acid complexes such as Cu(II) bis-L-valine up to 900 nm on improved instrumentation installed since the work reported here was completed gives no evidence of a shoulder on the positive peak at 765 nm.[27] The shoulders observed[11] may be artifacts due to xenon arc lines. Thus only one band appears at wavelengths longer than 700 nm and the simple course suggested above seems adequate.

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