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COPPER COMPLEXES OF Di-, Tri-, AND TETRA-PEPTIDES CONTAINING TRYPTOPHAN, HISTIDINE AND ARGININE

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Key Words: Copper complexes; Tryptophan, Histidine and Arginine peptides.

ABSTRACT

Fifty Seven copper complexes of di-, tri-, and tetra-peptides containing tryptophan, histidine and arginine are studied spectrophotometrically. The λ_{max} and colour of the complexes are dependent on the sequence of the amino acid in the dipeptide methyl esters of tryptophan and arginine; and independent on the sequence of dipeptides of histidine or in any of the tri- and tetra-peptides of histidine, arginine and tryptophan. The results achieved confirmed that the nitrogen atoms of the indole nucleus in tryptophan, imidazole ring in histidine and guanidino group in arginine do not participate in complex formation of all studied di-, tri- and tetra-peptides. However, the amide group and the hydrazide group of dipeptide amides and dipeptide hydrazides participate in complex formation.

INTRODUCTION

Poddubnaya and El-Naggar (1966, 1967) have investigated the copper complexes of a series of di-, tri- and hexapeptides containing lysine, ornithine and serine. El-Naggar *et al* (1973) studied a series of penta-, hexa- and heptapeptides and some hydrazides containing threonine, serine, lysine and diaminobutyric acid (Salem *et al* 1974 and El-Naggar *et al*, 1976).

The present investigation involves studies of the copper complexes of some di-, tri- and tetrapeptides (I-LVII) containing tryptophan, histidine and arginine with different amino acid sequences.

EXPERIMENTAL PROCEDURES

The present investigation involves studies of the copper complexes of some di-, tri- and tetrapeptides (I-LVII) containing tryptophan, histidine and arginine with different amino acid sequences.

Preparation of copper complexes

A 0.01 M Solution of the peptide in 1N KOH in absolute methanol (4 ml) was introduced into 5 ml measuring flask. The mixture was brought to the mark by addition of a 0.04 M solution of copper acetate in water. The complex solution was centrifuged, filtered off and the clear solution examined using a spectrophotometer. The absorption curves of the peptides are given in Fig. 1, λ_{max} and the absorbances of the peptides are listed in Table 1.

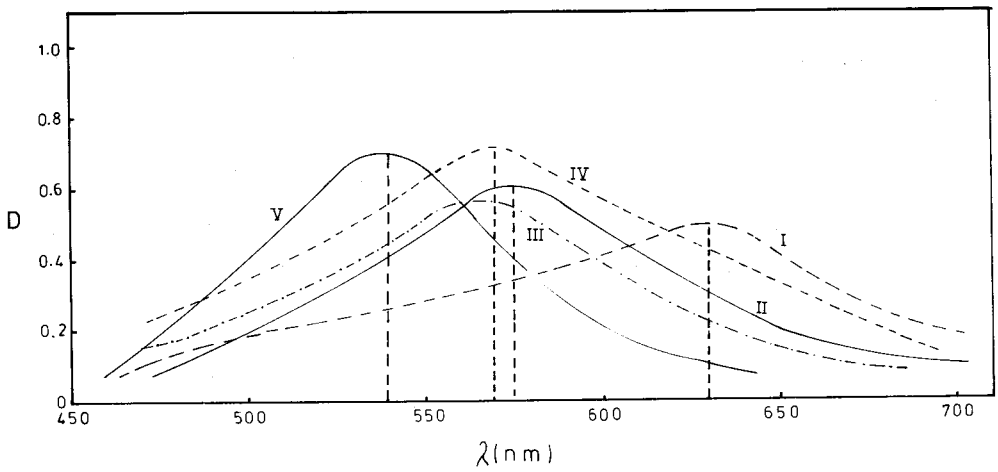


Figure 1 Copper complexes of some di, tri and tetra peptides of :

I. Tos - Val - His - OMe
II. Tos - Val - His - NH_2
III. Tos - Val - His - N_2H_3
IV. Tos - His - Gly - Gly - OMe
V. Tos - (Gly_3) - His - OMe

Determination of the Cu:peptide ratio

First, isomolar solutions of the peptide and the copper salt were prepared from a 0.003 M solution of the peptide in absolute methanol and a 0.003 M solution of $CuCl_2$. $2H_2O$ in absolute methanol, which were mixed to give the following proportions of the components : 0.5:4.5; 1.4; 1.5:3.5; 2:3; 2.5:2.5; 3:2; 3.5:1.5; 4:1; 4.5:0.5. To each mixture 1 ml of aqueous KOH was added and the mixture was stirred and filtered and the absorbance measured at λ_{max}. From the results, diagrams were constructed showing the relation between absorbance and the Cu:peptide ratio (cf. Fig. 2 and Table 1). All spectrophotometric measurements are performed using a Unicam SP 8000 spectrophotometer.

RESULTS AND DISCUSSION

Previous studies (Poddubnaya and El-Naggar 1966, 1967 and El-Naggar *et al* 1973, 1976 and Zewail 1974) revealed that the position of λ_{max} of the copper complexes of the di-, tri-, penta- and hexapeptide methyl esters depends on the order and structure of the amino acids in the peptide.

Our experimental data showed that protected N-tosyldipeptide methyl esters containing tryptophan residues (I-VI), histidyl and histidine residues (XIV-XX) and arginine residues (XXIII - XXVI) have maximum absorption characteristic of normal N-tosyldipeptide methyl esters of alanine, glycine, serine (Poddubnaya and El-Naggar, 1966, 1967) and valine (VII)

Table 1

Copper complexes of di-, tri- and tetrapeptides containing tryptophan, histidine and arginine.

Compd. No.	Name of the peptide*	Colour of complex	λ_{max} (nm)	Absor-bance	Cu: peptide ratio
I	Tos-Gly-L-Try-OMe	Blue	620	0.320	1:1
II	Tos-L-Ala-L-Try-OMe	Blue	620	0.460	1:1
III	Tos-β-Ala-L-Try-OMe	Blue	610	0.125	--
IV	Tos-L-Val-L-Try-OMe	Blue	610	0.385	1:1
V	Tos-L-Leu-L-Try-OMe	Blue	615	0.287	1:1
VI	Tos-L-Try-L-Try-OMe	Blue	610	0.245	1:1
VII	Tos-L-Val-L-Val-OMe	Blue	625	0.420	1:1
VIII	Tos-L-Try-Gly-OMe	-ve	-ve	---	--
IX	Tos-L-Try-L-Val-OMe	-ve	-ve	---	--
X	Tos-L-Try-DL-Ser-OMe	-ve	-ve	---	--
XI	Tos-L-Try-DL-Phe-OME	-ve	-ve	---	--
XII	Tos-2-Aba-L-Try-OMe	-ve	-ve	---	--
XIII	Tos-3, 4 Di (OH)-Phe-L-Try-OMe	-ve	-ve	---	--
XIV	Tos-Gly-L-His-OMe	Blue	640	0.730	1:1
XV	Tos-L-Val-L-His-OMe	Blue	630	0.145	1:1
XVI	Tos-L-Leu-L-His-OMe	Blue	630	0.125	1:1
XVII	N^{α}, N^{im}-Di Tos-L-His-Gly-OMe	Blue	610	0.185	1:1
XVIII	N^{α}, N^{im}-Di Tos-L-His-L-Val-OMe	Blue	630	0.150	1:1
XIX	N^{α}, N^{im}-Di Tos-L-His-L-Leu-OMe	Blue	615	0.230	--
XX	N^{α}, N^{im}-Di Tos-L-His-L-His-OMe	Blue	620	0.720	1:1
XXI	Tos-2-Aba-L-His-OMe	-ve	-ve	---	--
XXII	Tos-3, 4-Di (OH)-Phe-L-His-OMe	-ve	-ve	---	--
XXIII	Tos-Gly-N^{G}-nitro-L-Arg-OMe	Blue	625	0.420	1:1
XXIV	Tos-L-Val-N^{G}-nitro-L-Arg-OMe	Blue	625	0.370	1:1
XXV	Tos-L-Leu-N^{G}-nitro-L-Arg-OMe	Blue	620	0.425	1:1
XXVI	N^{α}, N^{G}-Di Tos-N^{G}-nitro-L-Arg-N^{G}-nitro-L-Arg-OMe	Blue	625	0.530	1:1
XXVII	N^{α}, N^{G}-Di Tos-N^{G}-nitro-L-Arg-Gly-OMe	-ve	-ve	---	--
XXVIII	N^{α}, N^{G}-Di Tos-N^{G}-nitro-L-Arg-L-Val-OMe	-ve	-ve	---	--
XXIX	N^{α}, N^{G}-Di Tos-N^{G}-nitro-L-Arg-L-Leu-OMe	-ve	-ve	---	--

Table 1 (Contd.)

Compd. No.	Name of the peptide*	Colour of complex	λ_{max} (nm)	Absor-bance	Cu: peptide ratio
XXX	Tos-2-Aba-N^G-nitro-L-Arg-OMe	-ve	-ve	---	--
XXXI	Tos-3, 4-Di (OH)-Phe-N^G-nitro-L-Arg-OMe	-ve	-ve	---	--
XXXII	Tos-L-Ala-L-Try-NH_2	Violet	555	0.542	1:1
XXIII	Tos-L-Val-L-Try-NH_2	Violet	575	0.350	1:1
XXXIV	Tos-L-Leu-L-Try-NH_2	Violet	560	0.159	1:1
XXXV	Tos-L-Try-L-Try-NH_2	Violet	555	0.750	1:1
XXXVI	Tos-L-Val-L-His-NH_2	Violet	575	0.560	1:1
XXXVII	Tos-L-Leu-L-His-NH_2	Violet	575	0.850	1:1
XXXVIII	Tos-L-Val-N^G-nitro-L-Arg-NH_2	Violet	560	0.360	1:1
XXXIX	Tos-L-Leu-N^G-nitro-L-Arg-NH_2	Violet	590	0.400	1:1
XL	Tos-L-Val-L-Val-NH_2	Violet	565	0.450	1:1
XLI	Tos-L-Val-L-Val-N_2H_3	Violet	570	0.310	1:1
XLII	Tos-L-Val-L-His-N_2H_3	Violet	570	0.350	1:1
XLIII	Tos-Gly-L-His-N_2H_3	Violet	565	0.250	1:1
XLIV	Tos-Gly-N^G-nitro-L-Arg-N_2H_3	Violet	570	0.450	1:1
XLV	N^{α}, N^{im}-Di-(Tos-Gly-Gly)-L-His-OMe	Violet	590	0.620	1:1
XLVI	Tos-L-Val-L-Val-L-Try-OMe	Violet	580	0.350	1:1
XLVII	Tos-L-Try-L-Val-L-Try-OMe	Violet	585	0.440	1:1
XLVIII	Tos-L-Val-L-Try-L-Try-OMe	Violet	580	0.310	1:1
XLIX	Tos-L-Val-L-Try-L-Val-OMe	Violet	585	0.480	1:1
L	Tos-L-Try-L-Val-L-Val-OMe	Violet	590	0.630	1:1
LI	Tos-L-Try-L-Try-L-Try-OMe	Violet	585	0.450	1:1
LII	Tos-L-Try-L-Try-L-Val-OMe	Violet	590	0.650	1:1
LIII	N^{α}, N^{im}-Di Tos-L-His-Gly-Gly-OMe	Violet	570	0.390	1:1
LIV	Tos-Gly-Gly-N^G-nitro-L-Arg-OMe	Violet	590	0.470	1:1
LV	Tos-Gly-Gly-Gly-L-Try-OMe	Red	540	0.640	1:1
LVI	Tos-L-Try-Gly-Gly-Gly-OMe	Red	535	0.550	1:1
LVII	Tos-Gly-Gly-Gly-L-His-OMe	Red	540	0.460	1:1

*Abbreviation are those proposed by IUPAC-IUB Commission on Biochemical Nomenclature., J. Biol. Chem., 250; 3215 (1975); 2-Aba = 2-aminobutyryl, 3, 4-Di (OH)-Phe = 3, 4-dihydroxyphenyl-alanyl. N^{im} = imidazole nitrogen, N^G = guanidino group, Tos = p-tosyl. -OMe = methyl ester, $-NH_2$ = amide and $-N_2H_3$ = hydrazide.

at λ_{max} 610-640 nm (cf. Figure 1 and Table 1). Moreover, it was found that the position of λ_{max} depends on the order of the amino acids in the dipeptide methyl esters. When the C-terminal amino acid in a dipeptide methyl ester is tryptophan or arginine and the N-terminal is glycine, alanine, valine, leucine, tryptophan or arginine, they form normal blue copper (II) complexes with λ_{max} 610-640 nm. When the N-terminal amino acid is tryptophan or arginine and the C-terminal is glycine, valine, serine or phenylalanine, the dipeptide methyl esters did not form Cu (II) complexes (cf. Table 1, Compounds VIII-XIII and XXVII-XXXI). However, all N-tosyldipeptide methyl esters containing histidyl and histidine residues (XIV-XX) form blue copper (II) complexes, λ_{max} 615-640 nm, characteristic of normal dipeptide methyl esters of valine (cf. Table 1).

Determinations of the compositions of the complexes by the Ostromyslenski (1911) - Job (1934) method showed that in all dipeptide methyl esters investigated the Cu:peptide ratio was 1:1 (cf. Figure 2 and Table 1). Hence, the suggestion of the participation of several copper atoms or peptide molecules in the complex formation had to be abandoned.

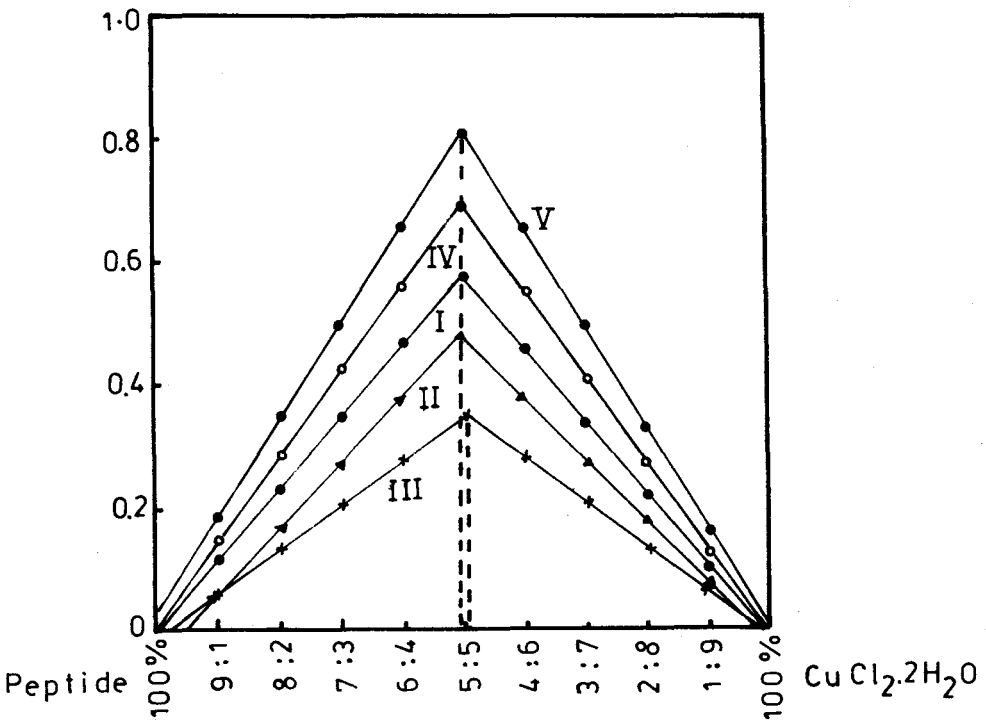


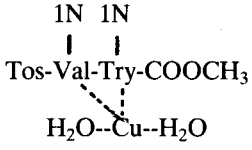
Figure 2 Diagram Cu : peptide ratios.

I. Tos - L - Val - L - Try - OMe at λ_{max} 610nm.
II. Tos - L - Val - L - Try - NH_2 at λ_{max} 575 nm.
III. Tos - L - Val L - L - His - N_2H_3 at λ_{max} 565nm.
IV. Tos - L - Val - L - Val - L - Try - OMe at λ_{max} 580nm.
V. Tos - Gly - Gly - Gly - L - Try - OMe at λ_{max} 540nm.

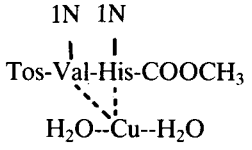
It is evident that the specific features of the complex formation of the dipeptide methyl esters of polyfunctional amino acids are associated with the internal structure of the complex and not its composition. For example, in the case of Tos - Gly - Try - OMe the dipeptide contains three nitrogen atoms capable of complex formation, Tos-Gly-His-OMe contains four nitrogen atoms. Tos - Gly - Arg-OMe contains five nitrogen atoms, and each contains a considerable number of oxygen containing groups. Hence, on the basis of the data available in the literature (Babko. 1955 and Plekhan, 1961), there are several possibilities for the formation of opper complexes with different structures:

1. The blue complex as of a typical dipeptide methyl ester with the participation of two nitrogen atoms only;
2. The violet complex as of a typical tripeptide methyl ester with the participation of three nitrogen atoms only;
3. The red complex as of a typical tetra- and higher peptides with the participation of four nitrogen atoms.

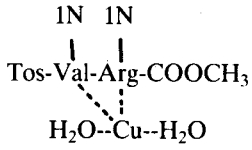
As stated above the dipeptide methyl esters containing C-terminal tryptophan or arginine residues and N- or C- terminal histidine residues form normal blue complexes with a Cu-peptide ratio 1:1, identical with the complexes of Tos - Gly - Gly - OMe and Tos - Val - Val - OMe. These results led to the conclusions that these complexes will have the schematic structures 1-3, with the participation of 2 $\propto$ - NH groups. Moreover, the presence of the additional nitrogen atoms of the indole nucleus in tryptophan, imidazole ring in histidine and guanidino group in arginine did not affect the colour, nature and composition of these complexes.



(Structure 1)



(Structure 2)



(Structure 3)

(blue Cu (II) complexes, λ_{max} 610-640 nm, Cu: peptide 1:1)

All dipeptide methyl esters containing 2-aminobutyryl or 3.4-dihydroxyphenylalanyl residues as the N-terminal amino acid in the dipeptide ester and the C-terminal amino acid is tryptophan, arginine or histidine did not form copper (II) complexes (cf. Table 1, Compounds XII, XIII, XXII, XXIII, XXXI, and XXXII).

The abnormal properties of some dipeptide methyl esters containing N-terminal 2-aminobutyryl-, 3,4-dihydroxyphenyl- alanyl-, arginyl- and tryptophyl residues may be attributed due to the steric effect, complex dimensions and stability of the complexes should be taken into consideration. Similar observations were reported in the case of some hippuryl-, trityl- and 2-aminobutyryl peptides (El-Naggar *et al*, 1977 and 1978).

Studies of the copper complexes of the dipeptide amides and dipeptide hydrazides of histidine, arginine and tryptophan show certain peculiarities when compared with the dipeptide methyl esters. For example: the copper complexes of Tos-Val-Try-amide, Tos-Val-Arg-amide and Tos-Val-His-amide and their corresponding hydrazides show maximum absorption characteristic for higher glycine and valine peptides (tri)) at $_{max}$ 555-590 nm (violet biuret reaction) (Plekhan, 1952, 1961). However, determination of the composition of the complexes by the Ostromyslenski (1911)-Job (1934) method showed that in all the dipeptide amide and dipeptide hydrazide complexes investigated the Cu: peptide ratio was 1:1 (cf. Figure 2 and Table 1. Complexes XXXII-XLIV). Thus the dipeptides Tos-Val-Try-NH_2, Tos-Val-His-NH_2 and Tos-Val-Arg-NH_2, contain four, five and six nitrogen atoms respectively and each of their corresponding hydrazides contains one more nitrogen atom and all these nitrogen atoms are capable of complex formation and there are again several posibilities for formation of copper complexes with different structures:

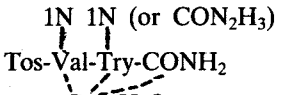
1. The violet complex as of a typical tripeptide methyl ester with the participation of three atoms of nitrogen (2 ∝ - NH and one NH of indole or imidazole or guanidino group);
2. All four or five or six nitrogen atoms may take part in complex formation with Cu; peptide ratio 2:1 (blue or violet complexes);
3. Four nitrogen atoms may participate with a Cu: peptide ratio 1:1 (red complex); and
4. Participation of the hydrazide group (-$CONHNH_2$) or the amide group (-$CONH_2$) in the complex (a violet complex as of a typical tripeptide methyl ester with the participation of 2 ∝ - NH groups and one NH of the amide (-$CONH_2$) or the hydrazide group (-$CONHNH_2$).

As stated above a violet copper complexes are formed with Cu: peptide ratio 1:1 and their λ_{max} 555-590 nm. It was found that when the second amino acid (C-terminal) (Try, His or Arg) in the dipeptide amide Tos-Val-Try-NH_2 or Tos-Val-Arg-NH_2 or in the dipeptide hydrazide Tos-Val-Try-N_2H_3 is replaced by valine (i.e. Tos-Val-Val-NH_2 or Tos-Val-Val-N_2H_3) no shift in λ_{max} was observed (cf. Table 1, complexes XXXII - XLIV). Hence, the inner structure of Tos-Val-Val - amide (or hydrazide) is identical to that of Tos - Val - Try - amide, Tos - Val - His - amide and Tos - Val - Arg - amide (or their corresponding hydrazides), it is almost definite that in these complexes with λ_{max} 550-590 nm and Cu: peptie ratio 1:1:, three nitrogen atoms take part in complex formation, two ∝ - NH amino groups and one N-atoms of the amide group (-$CONHNH_2$) or the hydrazide group (-$CONHN_2$) of the C-terminal amino acid.

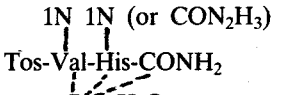
Comparative studied of the copper complexes of different dipeptide methyl esters and their corresponding dipeptide amides and hydrazides support the same conclusion (cf. Table 1, complexes I - XLIV).

From the results obtained it is evident that the indole nitrogen of tryptophan, imidazole nucleus of histidine and guanidino group of arginine does not participate in complex formation of dipeptide methyl esters and their corresponding amides and hydrazides.

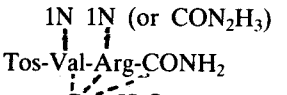
The dipeptide amide and hydrazide copper (II) complexes are suggested to have the schematic structures 4-6.



(Structure 4)



(Structure 5)



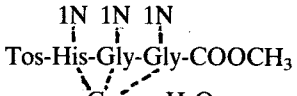
(Structure 6)

(Violet copper (II) complexes, λ_{max} 555-590 nm, Cu:peptide = 1:1).

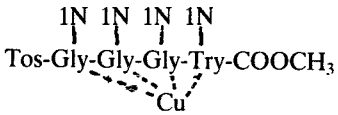
Studies of the copper complexes of different tripeptides containing N-or C-terminal and intermediate Try or Arg or His - residues show that all tripeptide methyl esters form normal violet copper (II) complexes. λ_{max} 560-580 nm, Cu : peptide ratio 1:1 (cf. Figs. 1,2 and Table 1, complexes XLV - LIV). For example : the N-tosyl tripeptide methyl ester of Val-Val-Try, Val-Try-Try, Try-Val-Try, Val-Try-Val, Try-Val-Val, Try-Try-Try, and Try-Try-Val gave Cu (II) complexes with the same λ_{max} and Cu: peptide ratio like that of normal tripeptides of glycine (Gly-Gly-Gly), valine and alanine (Plekhan, 1952, 1961).

In previous papers (Poddubnaya and El-Naggar 1966, 1967 and El-Naggar *et al* 1976, 1978), the participation of the N-terminal lysine or ornithine residues in tri- and hexapeptide complexes was confirmed and some violet or reddish-violet complexes were formed with Cu: peptide ratio 2:1.

Experimental results, showed that all the investigated tetrapeptides form red copper complexes with λ_{max} 530-550 nm, and their Cu: peptide ratio was 1:1 (cf. Table 1, complexes LV - LVII and Figures. 1,2). From these data it is confirmed that the indole nucleus of tryptophan, imidazole ring of histidine and guanidino group of arginine did not participate in complex formation of Di-, tri- and tetrapeptides even when Try-, Arg- or His-residues made the N- or C-terminal amino acid in the peptide. The tri- and tetrapeptide methyl ester Cu (II) complexes are suggested to have the schematic structures 7, 8.



(Structure 7, violet complex, λ_{max} 570 nm, Cu:peptide = 1:1)



(Structure 8, red complex, λ_{max} 530 nm, Cu:peptide= 1:1)

The difference in the nature and colour of the copper complexes of di-, tri- and tetrapeptides containing lysine, ornithine or diaminobutyric acid and tryptophan, arginine or histidine may be attributed to the difference in the length of the side chain and some conformational aspects should be considered.

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متراكبات النحاس للببتيدات الثنائية والثلاثية والرباعية المحتوية على التربتوفان والهستيدين والارجينين

أحمد محمد النجار ومحمود راغب زاهر وشريف أنور عبد الغفار

تضمن البحث دراسة سبكتروفوتومترية لسبعة وخمسين من متراكبات النحاس للببتيدات الثنائية والثلاثية والرباعية المحتوية على التربتوفان والهستيدين والارجينين .

واتضح من الدراسة أن طول الموجه القصوى ولون المتراكب يعتمد على ترتيب الاحماض الامينية في الاسترات الميثيلية للببتيدات الثنائية المحتوية على التربتوفان والارجينين وبينما لا يعتمد على ترتيب الاحماض الامينية فى الببتيدات الثنائية المحتوية على الهستيدين أو فى أى من الببتيدات الثلاثية والرباعية المحتوية على الهستيدين والارجينين والتربتوفان .

وأوضحت النتائج أن ذرات النيتروجين في مجموعة الاندول فى الارجنين أو فى مجموعة الاميدازول في الهستيدين أو في مجموعة الجوانيدين في الارجنين لا تشارك في تكوين المتراكبات للببتيدات الثنائية والثلاثية والرباعية التي تمت دراستها مع النحاس ـ وبينما ثبت أن مجموعة الاميد في أميدات الببتيدات الثنائية ومجموعة الهيدرازيد في هيدرازيدات الببتيدات الثنائية تشارك في تكوين متراكبات هذه الببتيدات مع النحاس .