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Theoretical study of dipeptide complexes of copper(II)

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Abstract

The L,L-Phe-Leu-Cu(II), L,L-Leu-Phe-Cu(II), L,L-Phe-Met-Cu(II), and L,L-Met-Phe-Cu(II) systems were studied using molecular modelling. The results obtained, which are in good agreement with results obtained by potentiometric studies show a significant increase in stability of copper complexes, when an aromatic residue is located in C-terminal (compared to the L,L-dipeptides containing the same amino acid residues), this phenomenon is attributed to the interaction between the d-orbital of copper and the π-electrons of the aromatic ring.
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1. Introduction

Copper is an essential element widely distributed in all the body of the mammals. Among the principal ions of transition metal in biology, Cu (II) is probably most effective in chelation with peptides at physiological pH [1]. In the body, the essence of copper is present under a non-labile form (for example: céruloplasmine). Labile copper is present in the form of complexes with amino acids or peptides [2].

Cu(II) forms very stable complexes with simple oligopeptides. The principal modes of coordination of the cupric ions with simple dipeptides are well established now [3].

At low pH, the species $[CuA]^+$ is formed with the dipeptide reacting like a bidentate ligand (I) (see Fig. 1). Towards pH 5, peptidic hydrogens can be deprotected in the presence of Cu(II), making it possible to rearrange the donors centres to form the structure (II) (see Fig. 1), which is the CuH−1A species. In the zone of high pH (>9), one of the molecules H_2O dissociates to form a mono-hydroxylic complex whose formula is $[CuH_{-2}A]^-$ or $[CuH_{-1}(OH)A]^-$, which has the structure (III) shown in Fig. 1. In addition to the monoligands, the structures of the complexes biligands are also well established. These complexes include CuA2 (IV) in Fig. 1.

Sigel et al. [1] indicate that there are great differences between the cupric complexes of the diglycine and the complexes with dipeptides where the residue glycyl is monosubstituted by coordinating side chains or not. A significant reduction in stability was observed in the cupric complexes with the dipeptides where the residue glycyl was substituted.

In agreement with Rabin [4], Sigel et al. conclude that stability increases with the lengthening of the side chains. This phenomenon was explained by the interactions between the non-covalent side chains [5,6]. Moreover certain authors propose the existence of interaction π–d between the aromatic ring of the side chains and the metal ions in certain complexes [7,8].

Potentiometric studies [11] (see Table 1) on the mixed complexes of Cu(II) in the presence of several amino acids [9,10] showed that the most stable species are formed when one of the ligands is aliphatic and the other one is aromatic (for example: phenylalanine or tyrosine) [11,12].

All the dipeptides used in this study are of L,L conformation. The equilibria in metal ion–dipeptide systems and the

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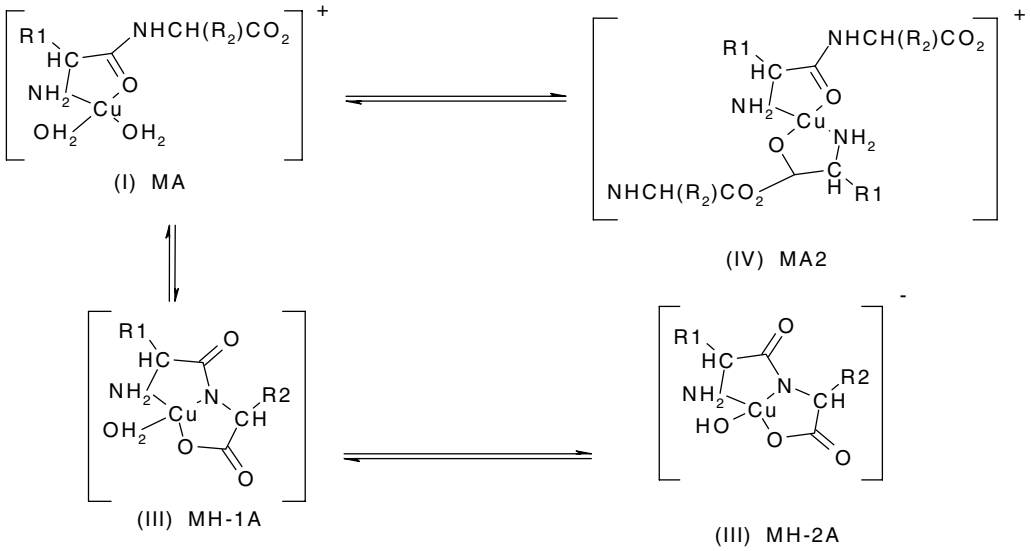


Fig. 1. Structural models of the cupric complexes with simple dipeptides.

Table 1
Reference bond length l_0 for Cu–ligand (Cambridge Structural Database 1995)

Atoms chaining	l_0 (angstroms Tot. Obs).	
Cu—O (sp^3) axial	2.39 (2.38)	1117
Cu—O (sp^2) equatorial	1.96 (1.94)	1162
Cu—O (sp^3) equatorial	(2.03)	
Cu—O (sp^2) axial	(2.15)	
Cu—N (sp^3) équatorial	2.00 (2.04)	2279

Values of reference bond length in brackets are taken from literature [25].

stability constants of complexes are defined by the following equations (charges are omitted for simplicity):

$$pM + rA + qH \overset{pqr}{\rightleftharpoons} M_pH_qA_r$$

$$\beta_{pqr} = [M_pH_qA_r]/[M]^p[H]^q[A]^r$$

where p, q, and r are the stoichiometric numbers. A negative value of q denotes a deprotonated or hydroxylated form of the species considered. The conjugated base of the ligand is noted A. HA is the zwitterions form, and M the metal ion.

2. Theoretical approach

Molecular modelling is used to study the influence of the side chains in the dipeptides, and especially those which contain a residue having an aromatic ring. We were interested to study the complexation of the Cu^{2+} close to the physiological pH, where the species (II) $MH_{-1}A$ is most predominant, with the four dipeptides according to: L,L-phenylalanyl-leucine (L,L-Phe-Leu), L,L-leucyl-phenylalanine (L,L-Leu-Phe), L,L-phenylalanyl-methionine (L, L-Phe-Met), and L,L-methionyl- phenylalanine (L,L-Met-Phe). Each dipeptide contains an aromatic side chain and an alkyl.

The $MH_{-1}A$ species is studied with the program EMO (Energy of MOlecule) follow-up by semi empirical calculations by the SAM1/d method, in both two cases:

The phenyl group is located in C-terminal.
The phenyl group is located in N-terminal.

EMO was developed by Blaive in 1993 [13] to treat the organic molecules, it includes three menus:

- Menu1: entry of the molecule using the keyboard; each atom is codified according to its hybridization. The maximum number of atoms is 420, hydrogen included.
- Menu2: geometrical handling on the molecule.
- Menu3: minimization of energy by molecular mechanics; it is done without derivation of the function energy. The version of this program placed at our disposal is designed to function on PC [14–17].

This program uses the force field MM2, which is the force field of Allinger [18], which was conceived at the beginning for the simple molecules (alkanes, carbonyl compounds, sulphides, amines, etc.). It is used to treat increasingly complex molecules.

The retained parameters, concerning structures of our complex ligands, are those proposed by the professor Blaive completed by the statistical study done at the level of the CSD (Cambridge Structural Database 1995).

Several effects are often set forth in the interpretation of experimental results [19]: (a) side-chain donor effect; (b) hydrophobic side chain–side chain interaction; (c) steric effects between the side chains; (d) effects from the surrounding solvent sphere. Another effect was proposed by

authors for copper(II)–dipeptide, nickel(II)–dipeptide, complexes containing aromatic side-chains [20,21]. It has been shown that the presence of an aromatic residue in a C-terminal site increases the stability of complexes.

- ***van der Waals radius of copper(II)***: Parameters used in this program are inspired from the work of Benmenni [22] who took the linear correlation between van der Waals radius published by Allinger, and those determined by Bondi [23] established by Bouraoui [15] who calculated constants in the case of iron. $r*(Allinger,MM2) = 0.856r(Bondi) + 0.058$ this term of Allinger informs us that van der Waals radius used in molecular mechanics can take different values on the basis of crystallographic results. One took values of the copper(II): ($r*Cu^{2+} = 1.90$ and $\varepsilon Cu^{2+} = 0.06$, calculated by Bouraoui, another value of $r* = 1.70$ that comes from data of the MOLCAD program developed by Brickman [24].
- ***Modelling of the Cu–Ligand interactions***: For reference bond length, the Cu—N and Cu—O bonds differ according to the type of atom of oxygen or nitrogen linked to the copper.We interrogated the CSD. We did a statistical study on more than 1000 cases having the same configuration of chromophor that our complexes. We chose values by taking the average values of those met at the time of this statistical study (see Table 1).
- ***Calculations of constant k***: Some measurements executed on the copper complexes, permitted to identify the frequency of the vibration γ (Cu–O) between 200 and 500 cm^{-1}. That permitted to evaluate by calculation the force constant k by the relation: $\gamma = 1/2\pi(k/m)^{1/2}$ and $k = 4\pi^2\ m\gamma^2$ with m (reduced mass): $1/m = 1/m_1 + 1/m_2.m_1$ and m_2 atomic masses of the oxygen and the copper, for the vibration Cu–O, we can consider that metal is fixed by the ligand that it carries, and the reduced mass will take the value of the oxygen: $1/m = 1/m^1 = 1/16$.After having minimized the energy of the various peptidic complexes of Cu(II), an optimisation of energy is carried out by the SAM1/d method. SAM1/d is a semiempirical method which takes in consideration d orbitals of transition metals.

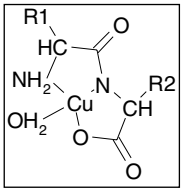
Table 2
Formation constants of copper(II)–dipeptide complexes at 25 °C, $I = 0.2$ mol dm^{-3} KNO_3 [11]

Dipeptide	$\log\beta_{1-11}$	$\Delta\log\beta_{1-11}$
L,L-Phe-Leu	0.93 ± 0.01	0.96
L,L-Leu-Phe	1.89 ± 0.01	
L,L-Phe-Met	1.61 ± 0.01	0.09
L,L-Met-Phe	1.70 ± 0.01	

$\Delta\log\beta_{pqr} = \log\beta_{pqr}$ (Φ C-terminal)$-$ $\log\beta_{pqr}$(Φ N-terminal).

3. Results

The study of the $MH_{-1}A$ species is interesting because it is the majority complex of the Cu(II)–dipeptide systems between pH 4.5 and 8 [11] i.e. close to the physiological pH. All the dipeptides used in this study are of L,L conformation.



Structure of $CuH_{-1}A$

All the structures studied in our work were built using the program EMO (version 2001) by introducing the Allinger code of the atoms of the molecules studied, by the keyboard of the computer then the energy is minimized by using the semi-empirical parameters. The most stable conformation is obtained from various starting geometries, after optimisation.

In order to avoid the local minima corresponding to unstable conformers, we carried out it with the option "SCAN" which makes it possible to sweep the surface of potential energy (PES). This enabled us to eliminate the geometries having little chance to generate the most stable conformers. Energies of the found conformers are optimised by the semi empirical method SAM1/d. The most stable conformations have the lower energies. For each structure we done the energies: E_{EMO}, $E_{stretching}$, $E_{bending}$, $E_{torsion}$, $E_{Van\ Der\ Waals}$, and $E_{SAM1/d}$.

The results (energies) obtained are gathered in Table 3.

4. Discussion

The formation constants of different species can be determined by the potentiometric measurements [11], see Table 2.

Table 3
Energies of the complexes calculated using EMO and SAM1/d

	L,L-Leu-Phe-Cu(II)	L,L-Phe-Leu-Cu(II)	L,L-Met-Phe-Cu(II)	L,L-Phe-Met-Cu(II)
E_{EMO} (kJ/mol)	66.711	67.506	60.011	63.445
$E_{stretch}$ (kJ/mol)	4.77	5.05	3.88	4.01
$E_{binding}$ (kJ/mol)	29.19	25.29	29.72	29.62
$E_{torsion}$ (kJ/mol)	−11.26	−9.78	−11.97	−10.23
E_{vdW} (kJ/mol)	44.02	46.95	38.39	40.05
$E_{SAM1/d}$(AMPAC)(eV)	−5153.092	−5153.081	−5191.404	−5191.336

Comparing each pair of ligands, where two amino residues are the same, but differing in sequence, we remark a significant increase in stability of complexes when the C-terminal residue contains an aromatic ring. The differences of formation constants are gathered in Table 2 ($\log\beta_{\text{L,L-Leu-Phe}} > \log\beta_{\text{L,L-Phe-Leu}}$ and $\log\beta_{\text{L,L-Met-Phe}} > \log\beta_{\text{L,L-Phe-Met}}$).

It is known that the influences of non-coordinating side chains are different according to the species. If we examine the structure of the $CuH_{-1}A$ species determined by e.s.r. measurements [26,27], we see that the two side chains are both involved in the stability of complex. Generally for L,L-dipeptides containing two large non-covalent side chain groups, the stability of $MH_{-1}A$ species is greater than of the corresponding L,D- or D,L dipeptides due to a hydrophobic interaction between two side chain groups which are located on the same side [5]. However, this hydrophobic interaction is less evident when the dipeptides contain a benzyl group, because the interaction between the alkyl group and the aromatic ring is less effective [5]. This increase in stability for the L,L-Leu-Phe and L,L-Met-Phe can be proposed as the result of a π–d interaction between the aromatic ring and the copper ion [28–30].

For every pair of dipeptides which contain the same amino acid residues, we could make the following comparison [12]:

(1) *Electronic effect*. For $MH_{-1}A$ species, this effect should be similar for the two dipeptides, because it is only dependent upon the distance. Referring the structural representation, R1 and R2 have almost the same distance from the center metal ion.
(2) *Steric effect*. This effect destabilizes the complex. The tridimensional model shows that the R2 residue sets above the metal ion. In the case of L,L-Leu-Phe and L,L-Phe-Leu, the two side chain $(CH_3)_2CHCH_2$ and $C_6H_5CH_2$ are different. But their steric effect may be comparable [5].
(3) *Hydrophobic interaction between two side chains*. The structure established for the $MH_{-1}A$ species according to the e.s.r. studies shows a $CuNO_2$ in-plane chromophore, which requires two side chains located on the same side, with respect to this basal plane when L,L-dipeptides are considered. Such an arrangement results in an interaction giving rise to hydrophobic micelles, thus decreasing the solvation enthalpy.

The above discussion excludes the possibility that the significant increase in stability, for the dipeptides containing an aromatic residue in C-terminal with respect to the N-terminal location, is the result of the other different effects than those enumerated in (1) to (3). This increase is then very probably due to π–d interaction.

In the light of the results obtained by calculation, we notice that the order of stability of the studied systems is as follows: L,L-Met-Phe-Cu(II) > L,L-Phe-Met-Cu(II) and L,L,Leu-Phe-Cu(II) > L,L-Phe-Leu-Cu(II).

We notice a significant increase in the stability of the cupric complexes, when the aromatic group is in C-terminal position (L,L-Leu-Phe and L,L-Met-Phe) compared to the dipeptide containing the same amino acids, but the residue phenyl is into N-terminal (L,L-Phe-Leu and L,L-Phe-Met), ($E_{\text{L,L-Leu-Phe}} < E_{\text{L,L-Phe-Leu}}$ and $E_{\text{L,L-Met-Phe}} < E_{\text{L,L-Phe-Met}}$). This phenomenon is attributed to the interaction between the π electrons of the aromatic ring and the d orbitals of the Cu(II). Results obtained by the calculation are in agreement with those obtained by the potentiomeric study ($\log\beta_{\text{L,L-Leu-Phe}} > \log\beta_{\text{L,L-Phe-Leu}}$ and $\log\beta_{\text{L,L-Met-Phe}} > \log\beta_{\text{L,L-Phe-Met}}$).

5. Conclusion

The presence of an aromatic ring in the C-terminal residue gives generally a more stable [$MH_{-1}A$] species. This stabilising effect was attributed to an interaction between the metal ion and the aromatic ring. This interaction is found only when the aromatic ring is located in the C-terminal residue, because the Cu—O bond is weak and easily distorted. However, the rigid Cu—N bond formed when the aromatic ring is in the N-terminal residue does not favour this interaction [11].

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