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Molecular Mechanics and Semi-empirical Analyses of the Interaction between Cu(II) and Amino Acids in Solution

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The interaction between Cu^{2+} and amino acids *viz.*, glycine, L-histidine and L-arginine, in solution has been investigated by UV-Visible spectrophotometry using the technique of continuous variation and molecular modeling. For each metal ion and amino acid combination, absorbance values at λ_{max} were plotted against concentrations of reactants. The maxima in the absorbance *vs.* concentration graphs gave the ratios at which the metal ions and amino acids combined. Copper(II) is found to form predominantly 1 : 3 complex with glycine, 1 : 4 complex with L-histidine and 1 : 2 complex with L-arginine. As expected, the *d-d* absorption bands due to copper(II) have been found to show a shift to shorter wavelengths when complexed with glycine, L-histidine and L-arginine. The proposed structures of the complexes have been optimized and their electronic spectra generated based on molecular mechanics and semi-empirical calculations. Comparison of the observed and predicted electronic spectra suggested the most likely structures present in solution.

Key Words: Copper(II), Glycine, Histidine, Arginine, Complexes, UV-Visible spectra.

INTRODUCTION

A number of metal ions perform diverse essential functions in living systems[1], although often unknown at the molecular level. Amino acids provide excellent donor centres for transition metal ions such as copper(II). Copper-amino acid complexes are a part of the accessible physiological pool of the element for most tissues[2]. Copper(II) complexes with amino acids and amino acid derivatives act as antiinflammatory, antiulcer, and/or radiation protection agents[3]. Copper(II) complexes with amino acids are also of fundamental importance as in such complexes copper(II) can act as a Jahn-Teller centre, adopting a number of coordination geometries from irregular square-planar, distorted planar, flattened tetrahedral, distorted tetrahedral, distorted square-pyramidal to distorted octahedral[4].

The interactions of metal ions with biomolecules belong to one of the most studied fields in bioinorganic chemistry. Metal ion-amino acid interaction has

been studied widely both in the solid state and in solution in water using a number of instrumental techniques[5-11]. But the works done on modeling the interaction between transition metal ions and amino acids in solution in water appear to be limited. Recently, Rulisek *et al.*[12] report on metal ion selectivity based on DFT calculations of interaction energies of amino acid side chains with selected transition metal ions.

As a part of our study to explore the interaction between metal ions and biological ligands, we report here the results of a study carried out on the interaction between Cu^{2+} and amino acids (AA) *viz.*, glycine (Gly), L-histidine (His) and L-arginine (Arg) using as a probe the effect of binding on the visible *d-d* transition of the metal ions (and in some cases the effect on the ultraviolet absorption spectrum of the amino acids) combined with molecular modeling. Essentially, our aim was to model the interaction between transition metal ions and amino acids in solution in water. In an earlier study, we used molecular modeling to explore the interaction between Ni^{2+} and nucleobases, nucleosides and nucleotides[13].

As a transition metal ion such as Cu^{2+} complexes, the ligand field causes splitting of its *d*-orbitals resulting into observable *d-d* transitions associated with the absorption of UV, visible or near infrared photons. The number of bands and their position on the spectrum are dependent on the symmetry and the strength of the ligand field. For example, in the octahedral ligand field, the five *d*-orbitals of a transition metal ion are split into two groups—three of one kind, equivalent to one another and labelled t_{2g}, and two of another kind, equivalent to each other, labelled e_g. The e_g orbitals are of higher energy than the t_{2g} orbitals. The difference in energy between the two groups of d-orbitals may correspond to the energy carried by a UV-Visible photon. Thus, UV-Visible spectrophotometry offers a simple technique to study the interaction between transition metal ions and various ligands including amino acids.

EXPERIMENTAL

Analytical grade copper(II) sulfate ($CuSO_4 \cdot 5H_2O$) was purchased from Ajax Chemicals, NSW, Australia. Amino acids were purchased from Sigma-Aldrich Pvt Ltd., NSW, Australia.

0 to 4 mL of 33.3 mM solutions of ($CuSO_4 \cdot 5H_2O$) and 100.0 mM amino acid (Gly, His or Arg), both made in milli-Q (mQ) water, were mixed in varying proportions and the total volume made up to 4 mL (Table-1). The solutions were placed in sealed plastic tubes and left at 22.0°C for 24 h. For each metal ion-amino acid combination, the UV-Visible spectrum from 190 to 900 nm of solution I (Table-1) was recorded using a Cary 1A UV-visible spectrophotometer, to determine the wavelength (λ_{max}) at which the absorbance was a maximum. A scan rate of 200 nm per min and a band width of 2 nm were used. The absorbance at λ_{max} was then measured for each solution A to S. In the case of L-histidine and L-arginine, milli-Q water was used as the blank whereas in the case of glycine reacting with Cu^{2+}, $CuSO_4$ blanks (at the same concentrations as in the corresponding solutions) were used. The absorbance values (given in Table-1) were

TABLE-1
INTERACTION BETWEEN 33.3 mmol L^{-1} Cu^{2+} and 100.0 mmol L^{-1} AA
(Gly, His or Arg): ABSORBANCE VALUES

Solution	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
mL M^{2+}	4.000	3.750	3.500	3.250	3.000	2.750	2.500	2.250	2.000	1.750	1.500	1.250	1.125	1.000	0.875	0.750	0.500	0.250	0.000
mL AA	0.000	0.250	0.500	0.750	1.000	1.250	1.500	1.750	2.000	2.250	2.500	2.750	2.875	3.000	3.125	3.250	3.500	3.750	4.000
[M^{2+}] mM	33.30	31.30	29.20	27.10	25.00	22.90	20.80	18.70	16.70	14.60	12.50	10.40	9.400	8.300	7.300	6.200	4.200	2.100	0.000
[AA] mM	0.000	6.300	12.50	18.80	25.00	31.30	37.50	43.80	50.00	56.30	62.50	68.80	71.90	75.00	78.10	81.30	87.50	93.80	100.0
n(AA)/n(M^{2+})	0.000	0.200	0.429	0.692	1.000	1.364	1.800	2.333	3.000	3.857	5.000	6.600	7.667	9.000	31.93	13.00	21.00	45.00	—
Abs at 755 nm[1]	0.000	0.058	0.092	0.111	0.146	0.157	0.170	0.183	0.196	0.184	0.176	0.162	0.154	0.142	0.116	0.110	0.070	0.032	0.007
Abs at 616 nm[2]	0.046	0.137	0.235	0.336	0.444	0.580	0.716	0.846	0.953	0.986	0.919	0.792	0.723	0.660	0.577	0.496	0.337	0.182	0.006
Abs at 617 nm[3]	0.044	0.149	0.268	0.403	0.571	0.792	1.042	1.069	0.950	0.832	0.714	0.595	0.536	0.473	0.414	0.355	0.237	0.124	0.008

[1] Cu^{2+} and Gly; [2] Cu^{2+} and His, [3] Cu^{2+} and Arg

then plotted against the added concentrations of the amino acid and Cu^{2+} to determine the stoichiometry of the formed adducts.

HyperChem Calculations

The proposed structures of the metal-amino acid complexes were optimized and their electronic spectra generated based on molecular mechanics and semi-empirical calculations using HyperChem 5 Molecular Visualization and Simulation program[14]. Geometry optimizations based on molecular mechanics (using MM^+ force field) and semi-empirical calculations (using ZINDO/1)[15] were used to find the coordinates of molecular structures that represent a potential energy minimum. For geometry optimization using both molecular mechanics and semi-empirical calculations, Polak-Ribiere routine with RMS gradient of 0.02 as the termination condition was used. To simulate the conditions in solution, the molecules were placed in a periodic box of TIP3P water molecules[16] followed by further cycles of geometry optimization. The actual dimensions of the box used and the maximum number of water molecules present are given in Table-2. The minimum distance between solvent molecules and solute atoms was set at 2.3 angstroms. Molecular dynamics calculations were used to obtain a lower energy minimum by enabling molecules to cross potential barriers[17]. The parameters used in simulated annealing were: heat time = 1 ps, run time = 0.5 ps, cool time = 0 ps, step size = 0.0005 ps, bath relaxation time = 0.1 ps, starting temperature = 100 K, simulation temperature = 300 K, temperature step = 30 K and data collection period = 4 time steps.

TABLE-2
DIMENSIONS OF PERIODIC BOX IN ANGSTROMS AND MAXIMUM NUMBER OF WATER MOLECULES

Molecule	X (Å)	Y (Å)	Z (Å)	Maximum number of water molecules
$Cu(Gly)_3(H_2O)_3$	18.70	18.70	18.70	216
$Cu(His)_4(H_2O)_2$	23.16	23.16	23.16	411
$Cu(Arg)_2(H_2O)_4$	26.57	26.57	26.57	264

For the optimized structures, electronic spectra were generated using the routine ZINDO/S following a singly excited configuration interaction (CI) calculation with semi-empirical method. HyperChem performs a self consistent field (SCF) calculation to obtain the reference electronic configuration associated with the ground state. Next, it generates a series of singly excited configurations, computes Hamiltonian matrix elements between them, and then diagonalizes the matrix to get the spectrum of the electronic states. The number of occupied and unoccupied orbitals set in the single point CI calculations was both set equal to ten.

TABLE-3
OBSERVED AND COMPUTED UV-VISIBLE SPECTRAL LINES

Complex	Observed λ_{max} values (nm)	Structure/Total energy/ΔH_f*	Predicted spectral lines (nm)
$Cu(Gly)_3$	A broad band ranging from 630 to 900 nm: maximum at 755	$Cu(Gly)_3(H_2O)_3$—bonded to carboxyl oxygens; –4537209.69; –4303408.73	676.6 (0.009); 637.8 (0.0004); 577.6 (0.003); 492.6 (0.0001); 473.4 (0.002); 437.5 (0.0002); 430.1 (0.0002); 417.9 (0.0001); 411.4 (0.002)
$Cu(His)_4$	A broad band ranging from 630 to 900 nm: maximum at 627	$Cu(His)_4(H_2O)_2$—bonded to imidazole nitrogens; –4537209.69; –4303408.73	688.5 (0.018); 582.7 (0.046); 566.7 (0.001); 542.4 (0.0004); 532.6 (0.0004); 529.9 (0.0003); 510.8 (0.01); 466.8 (0.0006); 461.3 (0.0007); 416.5 (0.052)
$Cu(Arg)_2$	A broad band ranging from 630 to 900 nm: maximum at 617	$Cu(Arg)_2(H_2O)_4$—bonded to NH_2 groups of the side-chains; –1026636.50; –863795.57	624.7 (0.0090); 612.6 (0.0030); 594.8 (0.0118); 530.5 (0.0287); 441.1 (0.054); 380.4 (0.0107)

*Energy values in kcal mol^{-1}.

RESULTS AND DISCUSSION

The plots of absorbance *vs.* concentration showed that Cu^{2+} formed mainly 1 : 3 complex with glycine (Fig. 1), 1 : 4 complex with L-histidine (Fig. 2) and 1 : 2 complex with L-arginine (Fig. 3).

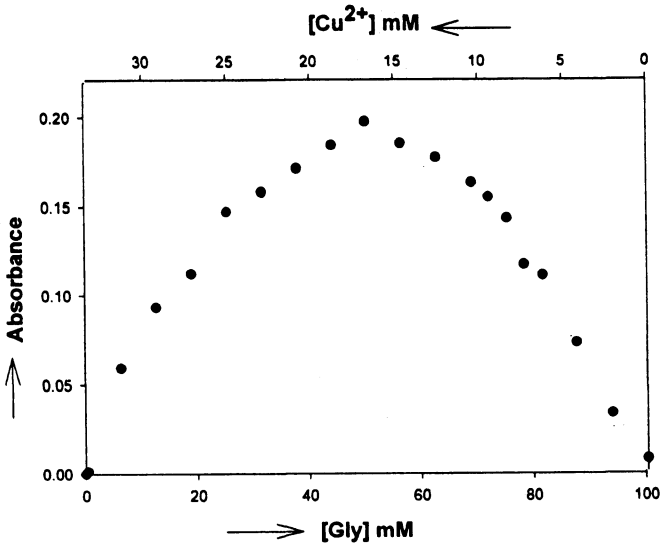

Fig. 1. Interaction between $CuSO_4$ and glycine

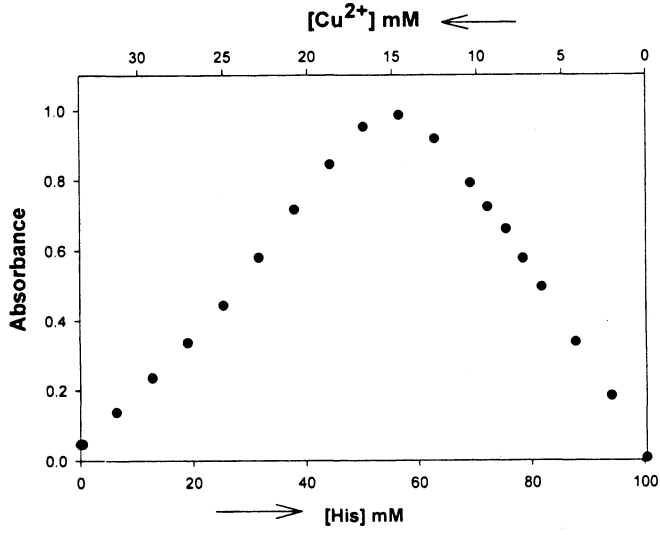

Fig. 2. Interaction between $CuSO_4$ and L-histidine

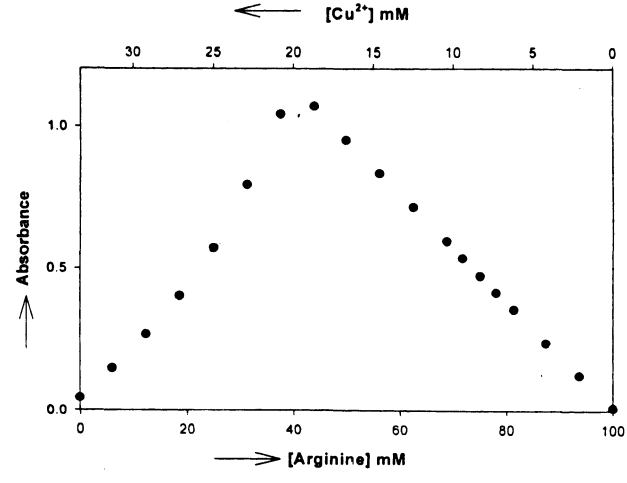

Fig. 3. Interaction between $CuSO_4$ and L-arginine

Gregory *et al.*[18] found that in presence of excess ligand, copper(II) formed 1 : 2 complex with arginine in solution at pH 5 to 9. For the Cu(II)-glycine complex, λ_{max} was found to be 755 nm and for the Cu(II)-L-histidine complex it was 616 nm, as compared to 818 nm for $Cu(H_2O)_6^{2+}$ (aq). All of the three complexes in solution were found to be darker blue in colour than $Cu(H_2O)_6^{2+}$ (aq). The results also show that both L-histidine and L-arginine are stronger ligands than glycine, causing a greater splitting of *d*-orbitals. This is in agreement with the observation that the stability constant for the complex of copper(II) with glycine is the smallest as compared to that of copper(II) with histidine or arginine[19]. For Cu(II)-glycine complex, the best agreement between the observed and predicted electronic spectra is found when copper(II) is considered to be coordinated to three carboxyl oxygens

and three water molecules so that the complex has the stoichiometry $Cu(Gly)_3(H_2O)_3$.

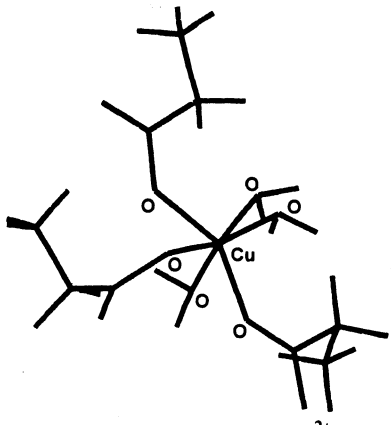

Fig. 4. Proposed structure of $Cu(Gly)_3(H_2O)_3$ in which Cu^{2+} is bonded to three glycine ligands through carboxyl oxygen and three water molecules

Since, it is well-known that glycine can act as a bidentate ligand, bidentate coordination of glycine to Cu(II) was also considered. However, when Cu(II) is considered to be chelated to three glycine ligands through carboxyl oxygens and amino nitrogens, the predicted spectral lines are found to be much shorter in wavelength than the observed values. In Cu(II)-L-histidine complex, best agreement between observed and predicted electronic spectra is found when copper is considered to be coordinated to four histidine ligands (through pyridine nitrogens of imidazole side chains) and two water molecules so that the complex has the stoichiometry $Cu(His)_4(H_2O)_2$. In this structure, there are two intramolecular H-bonds: one between a coordinated water molecule and the carboxyl oxygen of a histidine ligand and the other between amino group and carboxyl group of histidine

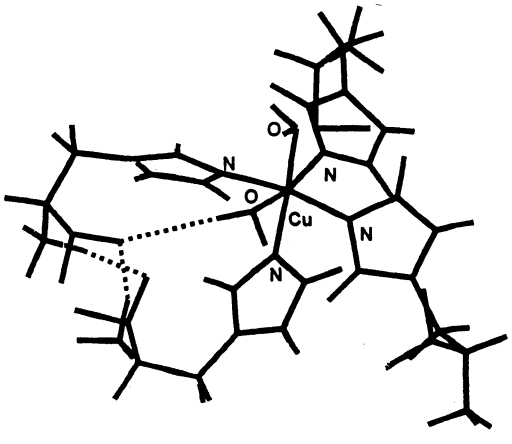

Fig. 5. Proposed structure of $Cu(His)_4(H_2O)_2$ in which Cu^{2+} is bonded to four histidine ligands through imidazole nitrogens and two water molecules

ligands. It should however be noted that histidine anion can act as a tridentate ligand when coordinated to transition metal ions such as Co^{2+}, Ni^{2+} and Cu^{2+}.

The structure gave a much better agreement between observed and predicted electronic spectra than other structures that would result if L-histidine was considered to be a polydentate ligand or if it was bonded to the metal ion through the carboxyl oxygen. In Cu(II)-L-arginine complex, best agreement between observed and predicted electronic spectra was found when copper was considered to be coordinated to 2 amino nitrogens of arginine side-chains and four water molecules so that the complex has the stoichiometry $Cu(Arg)_2(H_2O)_4$. In this structure, each of the two arginine ligands formed a macrocyclic ring through H-bonds.

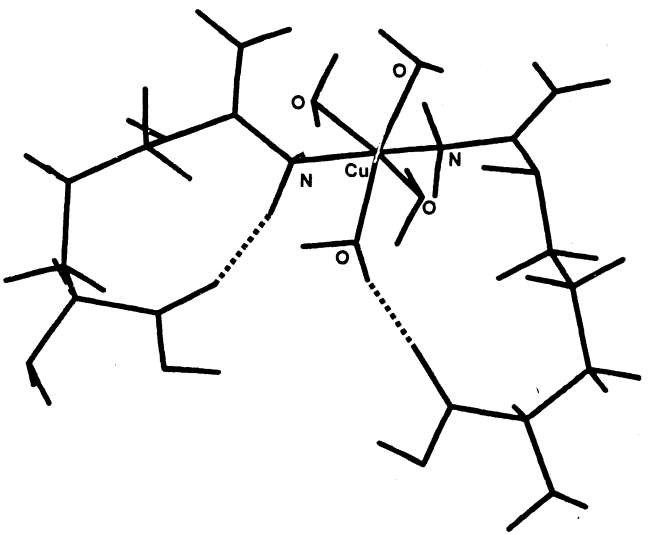

Fig. 6. Proposed structure of $Cu(Arg)_2(H_2O)_4$ in which Cu^{2+} is bonded to two ariginine ligands through side-chain nitrogens and four water molecules

In the structures all of the complexes (optimized by semi-empirical calculations), the copper ion was found to have a distorted octahedral geometry with four shroter bonds (ranging from 195 to 215 pm) and two longer bonds (of the order of 280 pm). However, when these structures were further optimized using molecular mechanics calculations again, the distortions disappeared. The d^9 configuration makes Cu^{2+} subject to Jahn-Teller distortion when placed in an environment of cubic symmetry[20]. When six-coordinate, the geometry is a severely distorted octahedron in which there are four short planer Cu—L bonds and two *trans* long ones. The generated electronic spectrum of $Cu(His)_4(H_2O)_2$ has a prominent absorption line at 583 nm as against the observed λ_{max} of 627 nm, that of $Cu(Gly)_3(H_2O)_3$ has a prominent absorption line at 677 nm as against the observed λ_{max} value of 755 nm and that of $Cu(Arg)_2(H_2O)_4$ has prominent absorption line at 531 nm as compared to the observed λ_{max} of 617 nm. It should be seen in all the three copper(II) complexes that the predicted λ_{max} values are to some extent shorter than the observed values. When Cu^{2+} was considered to be chelated to amino acids, generally it was found that the predicted lines for the optimized structures were even shorter than the λ_{max} values found in the observed spectra (results not shown).

For example, when Cu^{2+} was considered to be chelated to three glycine ligands, the optimized structure gave maximum absorbance at 352 nm was compared to the observed value of 755 nm. It may be noted that although semi-empirical calculations can give a satsifactory prediction of electronic spectra, the calculations fail to predict Jahn-Teller distortion for which better results are obtained from DFT calculations.

The above results show that among the three amino acids: Gly, His and Arg, His and Arg act as stronger ligands than Gly.

Conclusion

In solution, copper(II) was found to form predominantly 1 : 3 complex with glycine, 1 : 4 complex with L-histidine and 1 : 2 complex with L-arginine. Molecular modeling analysis suggested that in $Cu(Gly)_3$ complex, Cu^{2+} was bonded to three carboxyl oxygens of glycine ligands and three water molecules so that the complex has the stoichiometry $Cu(Gly)_3(H_2O)_3$; in $Cu(His)_3$ complex, it was bonded to four imidazole nitrogens and two water molecules so that the complex has the stoichiometry $Cu(His)_4(H_2O)_2$; and in $Cu(Arg)_2$ complex, it was bonded to two amino nitrogens of arginine side-chains and four water molecules so that the complex has the stoichiometry $Cu(Arg)_2(H_2O)_4$.

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