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A DFT study of copper(II) binding to glycyl-L-histidyl-L-Lysine tripeptide

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ABSTRACT

Density functional theory is employed to examine the interactions of copper(II) ion and glycyl-L-histidyl-L-lysine tripeptide in both gas phase and aqueous solution. Thermodynamic parameters, electronic structures, and bonding characteristics of the complexes are investigated by using the hybrid functional B3LYP in conjunction with the basis set 6-311++G(d,p). Computed results show that copper(II) ions prefer binding to the peptide via the glycine amino nitrogen, the deprotonated amide nitrogen of glycine–histidine peptide bond, the non-deprotonated amide nitrogen of histidine–lysine peptide bond, and the lysine carboxylate oxygen. The interaction is strongly dominated by electrostatic effects, namely hydrogen bond contributions.

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1 INTRODUCTION

The copper-binding glycyl-L-histidyl-L-lysine tripeptide, usually abbreviated as GHK, is naturally present in the human plasma, saliva, and urine (Schlesinger *et al*., 1977). The compound was first isolated from human plasma albumin by Pickart and Thaler (1973). Since its discovery, numerous studies have been devoted to its biochemical and biological functions. One of the most striking findings was its ability to stimulate the synthesis of collagen in skin fibroblasts, increasing the growth of cultured cells (Pickart, 1981). The molecule also plays a special role in the delivery of copper into the cells which is required for the cellular functions in the nontoxic form (Pickart *et al*., 1980). Subsequent studies confirmed that the peptide inherently has a high affinity to copper(II) ions, readily forming the stable chelate Cu-GHK (Rózga *et al*., 2007).

Although the biochemical and biological functions of the Cu-GHK complex have extensively been investigated (Pickart *et al*., 2017), its molecular structure is surprisingly not clearly elucidated to date. Several spectroscopic techniques have been utilized to decipher its molecular structure and some related properties (Hureau *et al*., 2011). However, some challenges for experiment remain because numerous possible metal binding sites of the peptide lead to great variety of conformations by altering energetic and steric constraints. Previous X-ray investigation conducted by Hureau *et al*. (2011) suggested a 3N+1O in-plane binding mode for Cu(II). Accordingly, the central atom is bound to the glycine amino nitrogen, the deprotonated amide nitrogen of the glycine–histidine peptide bond, the N(π) nitrogen of the imidazole side chain of the histidine, and the oxygen atom of lysine carboxylate. On the contrary, it is widely depicted that in the Cu-GHK complex, the copper(II) ion is coordinated by three different nitrogen atoms, namely, from the imidazole of histidine, the α-amino group of glycine, and the amide nitrogen of the glycine-histidine peptide bond (Pickart *et al*., 2012).

Furthermore, as compared to the well characterized biological significance of Cu-GHK complex, relatively less is known about its electronic and energetic properties. Some experimental data on thermodynamics of Cu^{2+} coordination to GHK could be found in the literature (Trapaidze *et al*., 2012), but quantitative atomic level information about the factors controlling the GHK-Cu interactions is still not unambiguous so far. As quantum chemical calculations have become an effective tool for analyzing electronic structures and related properties of transition metal compounds (Peverati and Truhlar, 2012), and have made significant contributions to profound elucidation on the interaction mechanisms between metal ions and biomolecules (Dasari *et al*., 2014), here DFT calculations are employed to examined in more details on the nature of these interactions of the copper(II) ion and the GHK tripeptide. This is in fact crucial to have a clear understanding on the activities of key molecules in living organisms such as the Cu-GHK complex.

2 COMPUTATIONAL METHODS

All calculations are carried out using the Gaussian 09 suite of program (Frisch *et al*., 2009). Geometries of the species considered are fully optimized without any symmetry or geometry constraints, making use of density functional theory (DFT) with the hybrid B3LYP functional, in conjunction with the full-electron basis set 6-311++G(d,p). Calculations are performed on the Cu-GHK complexes with no net charge, and a doublet multiplicity, because of the presence of one unpaired electron in the Cu^{2+} ion. The unrestricted formalism (UHF, UB3LYP) is employed for computations of the open-shell systems. Initial structures of the complex for geometry optimizations are generated by placing the Cu(II) cation in the vicinity of electron-rich centers, i.e. the N-terminal amino group of glycine, the deprotonated amide nitrogen of peptide bonds, the O atom of the lysine carboxylate, and the N(π) nitrogen in the histidine imidazole ring. Harmonic vibrational frequencies are calculated at the same level as the optimization procedure to confirm the character of optimized geometries as local minima on the potential energy surface and to estimate the zero-point vibrational energy (ZPE) corrections.

Zero-point and thermal enthalpies corrections are then employed to obtain free energies using the following equation (2):

$$\Delta G^0(298K) = \Delta E + \Delta ZPE + \Delta TCG \quad (2)$$

where ΔE is the relative electronic energy at 0 K, ΔZPE is the relative vibrational energy at 0 K, while ΔTCG is relative changes in Gibbs free energy in going from 0 to 298 K.

The metal-ligand binding energy E_b of the complex Cu-GHK is computed using the equation (3):

$$E_b = E_{GHK-Cu} - (E_{GHK} + E_{Cu}) \quad (3)$$

where E_{GHK}, E_{Cu}, E_{GHK-Cu} are total energies of GHK in its dianionic state, Cu^{2+} and Cu-GHK complex, respectively. As for a convention, a negative value of E_c corresponds to a stabilizing complexation. The greater the computed value of the binding energy is, the stronger the affinity of Cu^{2+} ion binding to the ligand is. Hence, this parameter can be used to evaluate the relative stability of the complex. The effect of solvent (aqueous solution) is simulated using the popular continuum model known as the integral equation formalism-polarizable continuum model (IEF-PCM) (Tomasi *et al*., 2005).

3 RESULTS AND DISCUSSION

3.1 Geometries and relative energies

The GHK molecule (Figure 1) exhibits several potential binding sites with the copper ion, i.e. nitrogen atoms from the amino groups of glycine and lysine, from the imidazole of histidine, from the amide groups of glycine-histidine and lysine-histidine peptide bonds, and oxygen atoms from the lysine carboxylate and peptide groups. At the B3LYP/6-311++G(d,p) level, six possible structures of its complex with copper(II) ion in the gas-phase are located. The optimized geometries and relative energies are presented in Figure 2. As for a convention, these structures are denoted as Cu-**GHK_N** in which N = 1 – 6 indicating the isomers with increasing relative energy (kcal/mol).

The conformation **Cu-GHK_1** (Figure 2), which is constructed by directly binding Cu-atom to one nitrogen atom of the glycine amino group, the nitrogen atoms of glycine–histidine and lysine–histidine peptide bonds, and one oxygen atom of lysine carboxylate group, is predicted to be the lowest-energy structure. Such an arrangement is relatively stabilized since the peptide moiety can form an internal hydrogen bond through the histidyl and amide groups.

Other less stable isomers including **Cu-GHK_2** and **Cu-GHK_3** (Figure 2), are almost energetically degenerate with a tiny energy gap of ~0.1 kcal/mol, and lie only 1.3 – 1.4 kcal/mol above the most stable isomer. In both **Cu-GHK_2** and **Cu-**

GHK_3 forms, the two amide nitrogen atoms of the peptide bonds are deprotonated, thus it is not allowed to form a hydrogen bond between the N(π) nitrogen of histidine and the H-atom of the lysine–histidine peptide bond. Instead, they are stabilized upon formation of a hydrogen bond between the H atom of amino and carboxyl groups of lysine.

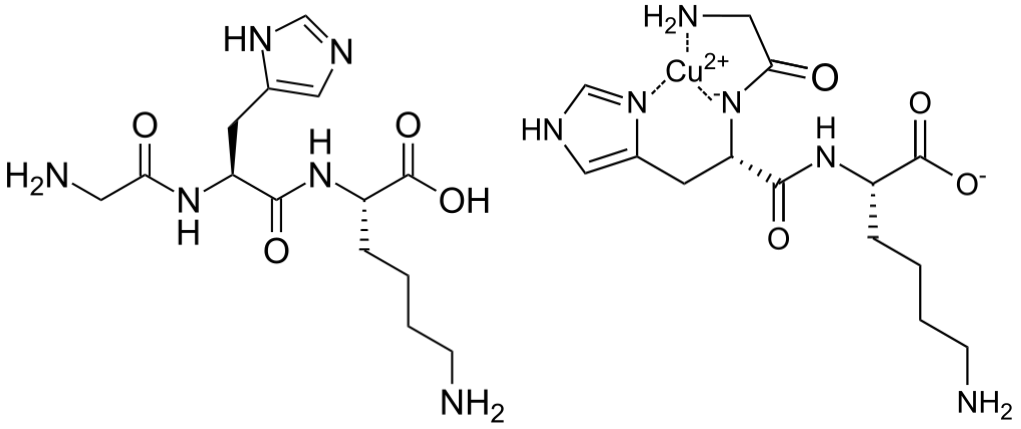
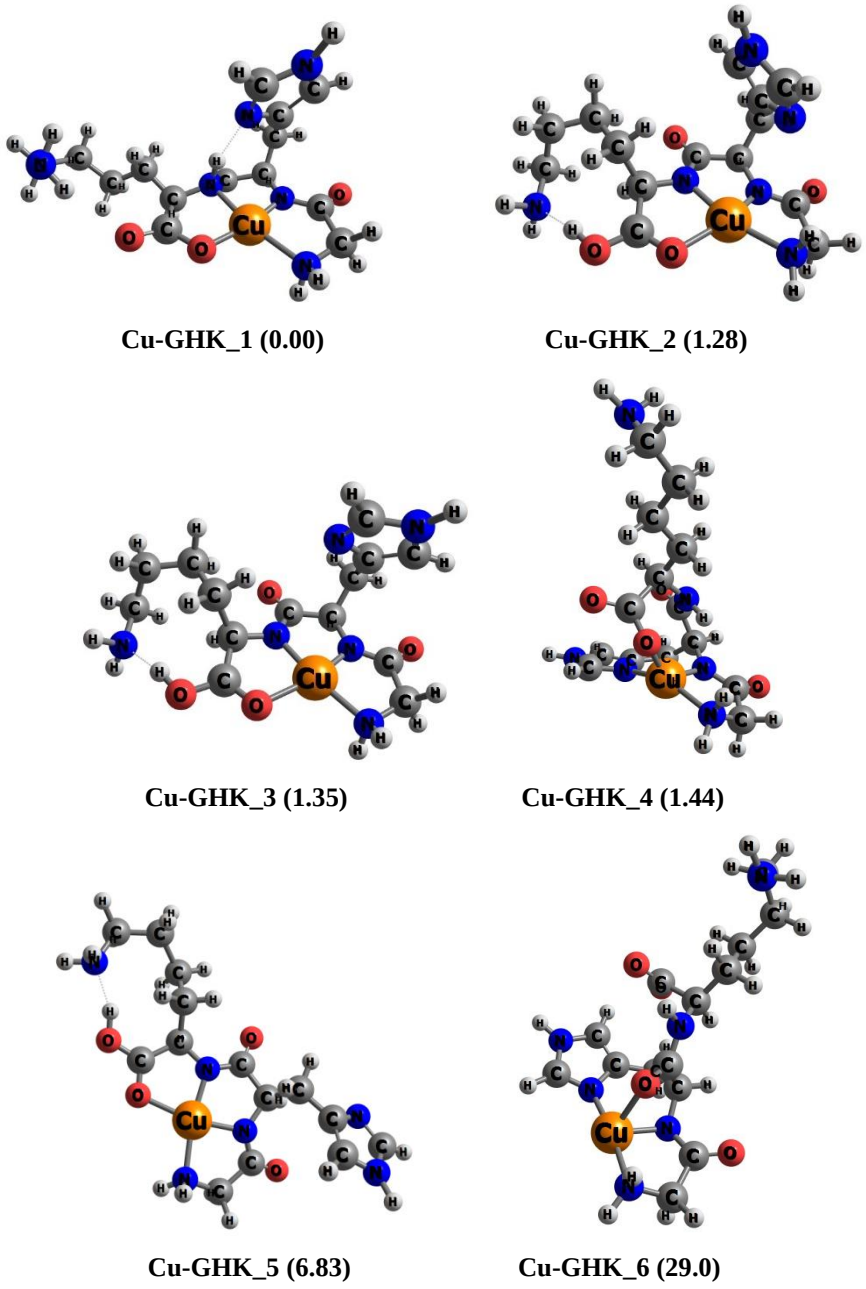


Fig. 1: Chemical formula of tripeptide GHK (left) and a proposed molecular structure of copper tripeptide Cu-GHK

Also stable is the isomer **Cu-GHK_4**, in which the copper ion is bound in a considerably distorted square planar geometry by the nitrogen of glycine amino group, the deprotonated amide nitrogen of the glycine–histidine peptide bond, the nitrogen of histidine and the oxygen of lysine carboxylate. At the level of theory used, this form is computed to be only around 1.5 kcal/mol higher in energy than **Cu-GHK_1**. Within the expected accuracy of the DFT methods, being ± 3 kcal/mol on molecular heats of formation, these forms are energetically degenerate and could emerge as competing to be the ground state.

The remaining isomer, i.e. **Cu-GHK_6** in Figure 2, is much less stable, being about 29 kcal/mol above **Cu-GHK_1**. It is formed by anchoring Cu-atom on the glycine amino nitrogen, the deprotonated amide nitrogen of the glycine–histidine peptide bond, the N(π) nitrogen of the histidine, and the oxygen of glycine–histidine peptide bond. The higher instability of this form can be understood by the fact that hydrogen bonds are not allowed in **Cu-GHK_1**. This observation indicates that hydrogen bond plays a key role on the interaction between copper ions and peptide fragments.

It should be noticed that all results mentioned above are investigated in vacuum. Calculations in the aqueous solution, however, reveal a different energy landscape as illustrated in Figure 3. In water solution, both isomers **Cu-GHKsol_1** and **Cu-GHKsol_2**, being separated from each other by only 0.1 kcal/mol, become thus the most stable conformations. They are stabilized thanks to a hydrogen bond between the H atom of amino and carboxyl group of lysine, as in **Cu-GHK_2** and **Cu-GHK_3**. The **Cu-GHKsol_3**, which is similar to **Cu-GHK_4**, is located to be 2.36 kcal/mol less stable than **Cu-GHKsol_1**. The isomer **Cu-GHKsol_4** with an internal hydrogen bond *via* the histidyl and amide groups is now 4.3 kcal/mol higher in energy than the ground state. Other local minimum, i.e. **Cu-GHKsol_5** in Figure 3, is around 11.5 kcal/mol above **Cu-GHKsol_1**. The peptide fragment in **Cu-GHKsol_5** serves as a tridentate chelator *via*three nitrogen atoms: glycine amino, amidyl and imidazole. Such a form has long been reported in the literature to be the best candidate for the molecular structure of GHK-Cu complex in aqueous solution (Pickart *et al*., 2012).

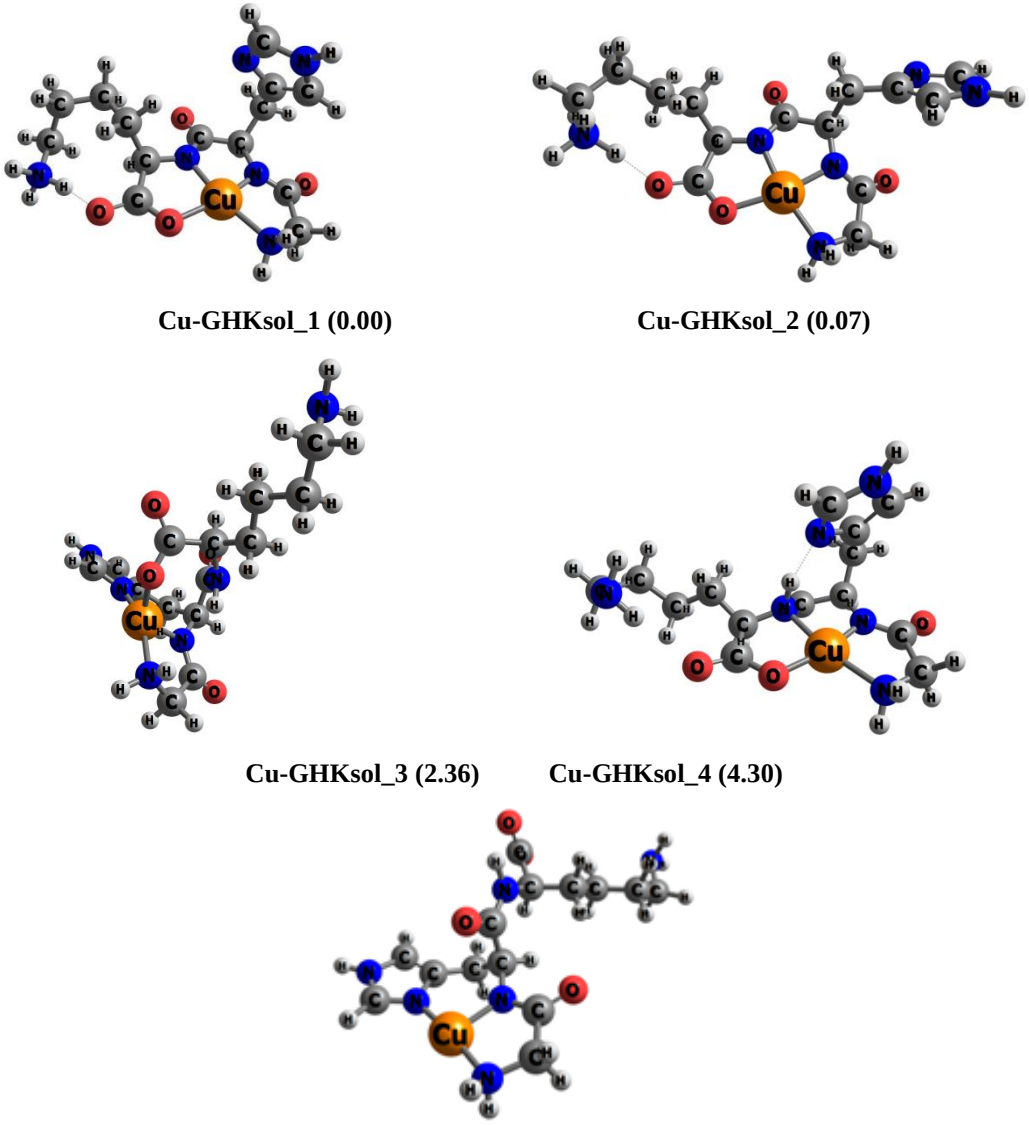


Cu-GHK_1 (0.00) **Cu-GHK_2 (1.28)**

Cu-GHK_3 (1.35) **Cu-GHK_4 (1.44)**

Cu-GHK_5 (6.83) **Cu-GHK_6 (29.0)**

Fig. 2: Optimized structures located for the GHK-Cu complex in vacuum. The values given in square brackets are their relative energies (UB3LYP/6-311++G(d,p) + ZPE, kcal/mol)



Cu-GHKsol_1 (0.00) **Cu-GHKsol_2 (0.07)**

Cu-GHKsol_3 (2.36) **Cu-GHKsol_4 (4.30)**

Cu-GHKsol_5 (11.5)

Fig. 3: Optimal structures located for the GHK-Cu complex in aqueous solution. The values given in square brackets are their relative energies (kcal/mol)

3.2 Energetics and chemical bonding

In order to examine the thermodynamic stability of the complex Cu-GHK, the metal-ligand binding energy, enthalpy and Gibbs energy of complexation reactions are calculated in both vacuum and aqueous solution. These energy changes in gas-phase (g) and in aqueous solution (aq) are described in the following processes:

$Cu^{2+}(g) + GHK(g) \rightarrow Cu\text{-}GHK(g) + 2\ H^{+}(g)$

$Cu(H_2O)_4^{2+}(aq) + GHK(aq) \rightarrow Cu\text{-}GHK(aq) + 2\ H^{+}(aq) + 4\ H_2O(aq)$

For the evaluation of solvation enthalpy and free energy, the experimental values are usually applied to the proton. Several values of the proton hydration enthalpy and free energy can be found in the literature. Here, the values $\Delta H_{hydration}$ and $\Delta G_{hydration}$ of H^+ are selected to be -269.4 and -259.5 kcal/mol (Lim *et al*., 1991), respectively. The gas-phase enthalpy $H_{H^+}(g)$ and the Gibbs free energy $G_{H^+}(g)$ of proton are taken to be the values of 1.47 and -6.30 kcal/mol (Fifen *et al*., 2014).

Table 1: Calculated values (kcal/mol) of binding energy (E_b), enthalpy (ΔH^{298}) and Gibbs energy (ΔG^{298}) for complexation reaction of Cu^{2+} and GHK in vacuum and aqueous medium

Complex	E_b	ΔH^{298}	ΔG^{298}	Complex	E_b	ΔH^{298}	ΔG^{298}
	In vacuum				In water		
Cu-GHK_1	40.0	41.4	38.1	Cu-GHKsol_1	-24.7	-23.3	-24.8
Cu-GHK_2	41.2	42.4	39.4	Cu-GHKsol_2	-24.6	-23.2	-24.3
Cu-GHK_3	41.4	42.5	39.8	Cu-GHKsol_3	-22.6	-20.6	-23.6
Cu-GHK_4	46.8	48.0	45.5	Cu-GHKsol_4	-20.5	-18.7	-21.0
Cu-GHK_5	67.7	69.4	65.8	Cu-GHKsol_5	-13.2	-11.0	-15.6

Table 1 presents the computed results in both gas phase and water environments. The high positive values of binding energy (E_b), enthalpy (ΔH^{298}) and Gibbs energy (ΔG^{298}) in vacuum indicate that the process is nonspontaneous because a large amount of energies is needed to eliminate two protons from the ligand. When the solvent effect is taken into account, these thermodynamic values are significantly lower, mainly due to the stabilization of charged species or polarized portions in the presence of solvent water molecules. For instance, the E_b value of the most stable complex changes from 40.0 for Cu-GHK_1 to -24.7 kcal/mol for **Cu-GHKsol_1**, in going from the gas phase to the water medium. A comparable trend is also observed for the enthalpy and Gibbs energy at 298 K (Table 1).

In aqueous solution, the values of binding energy, enthalpy and Gibbs energy of all complexes become much more negative, indicating that the process could be spontaneous, and the peptide naturally has a high affinity for the Cu^{2+} ion as pointed out previously. Under the effects of such a polar solvent, the spontaneity of the complexation reaction is not unexpected as the hydrogen ion releases a large amount of energy (-269.4 kcal/mol) when it dissolves in water. Current computed values of the free energy change given in Table 1 also agree well with experiment. Previously, the experimental values of ΔH^{298} and ΔG^{298} for the Cu-GHK complex in aqueous solution were determined to be around -22.5 kcal/mol (-94 kJ/mol) (Conato *et al.*, 2001).

The nature of interactions between the peptide and copper ion is analyzed on the basis of frontier orbitals in the resulting complexes. Let us take a closer look at **Cu-GHK_1** and **Cu-GHKsol_1** as typical examples. Quantitatively, the electronic structure of transition metal complexes can be attained by an analysis based on the ligand field theory with parameters adjusted to DFT calculations (LF-DFT) (Atanasov *et al.*, 2004). Accordingly, it is presumed that the molecular orbitals where the d electrons inhabit are mainly contributed from the transition metal d-functions.

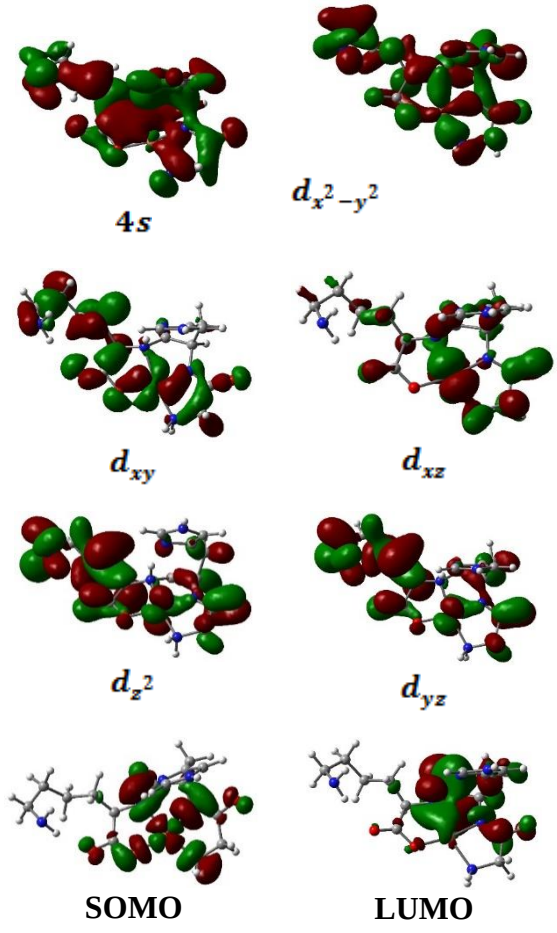


Fig. 4: Frontier MOs of Cu-GHK_1 complex derived from the interactions between valence orbitals of copper(II) ion and GHK peptide

Figure 4 shows the shapes of several higher-lying orbitals in the **Cu-GHK_1** complex. These orbitals are derived from the interactions between valence orbitals of copper and those of GHK peptide. Inevitably, the Cu-GHK has an open-shell ^2A ground state, corresponding to an unpaired electron on the $d_{x^2-y^2}$ orbital of Cu^{2+} (d^9) ion in a square-planar ligand field. As illustrated in Figure 4, this singly

occupied orbital (SOMO) is an anti-bonding orbital and composed mainly of the $d_{x^2-y^2}$(Cu^{2+}). The lowest-unoccupied orbital (LUMO) has a non-bonding character and receives a much greater contribution from the ligand orbital. The interactions that form chemical bonding in the complex occur between the $4s$, $d_{x^2-y^2}$, d_{xy} and d_{xz} of Cu^{2+}, and the same symmetry counterparts of GHK, giving rise to three σ and one π bonding orbitals (Figure 4). Similar mode of orbital interactions is also observed for the **Cu-GHKsol_1** complex in aqueous solution.

4 CONCLUDING REMARKS

In the present theoretical study, a thorough investigation on the interactions between copper(II) ion and glycyl-L-histidyl-L-lysine tripeptide was performed in both gas phase and aqueous solution using quantum chemical calculations. The density functional B3LYP combined with a full electron basis set (6-311++G(d,p)) was employed to examine geometric parameters, thermodynamic parameters, electronic structures, and bonding characteristics of the resulting complexes.

Computed gas phase results show that copper(II) prefers a square-planar coordination, binding to the peptide via the glycine amino nitrogen, the deprotonated amide nitrogen of glycine–histidine peptide bond, the non-deprotonated amide nitrogen of histidine–lysine peptide bond, and the lysine carboxylate oxygen. This conformation is greatly stabilized owing to a strong H-bond interaction between the hydrogen atom on amide group of histidine–lysine peptide bond and the N(π) nitrogen of the imidazole side chain.

Following solvation effects, both hydrogen atoms on amide groups are deprotonated, and thus the stabilization of the resulting complex is due to the hydrogen bond between the H atom of amino and carboxyl group of lysine. In aqueous medium, the peptide moeity could alsoserve as a tridentate chelator *via* three nitrogen atoms, namely, the glycine amino nitrogen, the deprotonated amide nitrogen of glycine–histidine peptide bond, and the N(π) nitrogen of the imidazole ring, but the resulting complex is predicted to lie about 11.5 kcal/mol higher in energy than ground state. When solvent effects are taken into account, the complexation process becomes more willing to occur with a Gibbs energy change of -25 kcal/mol. The central atom mainly uses $4s$, $d_{x^2-y^2}$, d_{xy} and d_{xz} orbitals for making chemical bonds with the peptide. Besides, the interactions are strongly affected by H-bond contributions.

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