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ORIGINAL PAPER

A quantum-chemical study of the binding ability of βXaaHisGlyHis towards copper(II) ion

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Abstract The present study analyzed binding of Cu^{2+} to tetrapeptides in water solution at several levels of theoretical approximation. The methods used to study the energetic and structural properties of the complexes in question include semiempirical hamiltonians, density functional theory as well as ab initio approaches including electron correlation effects. In order to shed light on the character of interactions between Cu^{2+} and peptides, which are expected to be mainly electrostatic in nature, decomposition of interaction energy into physically meaningful components was applied.

Keywords Density functional theory · Intermolecular interaction · Tetrapeptide · Copper binding

Introduction

Copper^{2+}-histidine species have been investigated extensively over the past four decades with an eye towards understanding of the role of Cu^{2+} in cell metabolism (see [1] and references therein). Copper ions have also been proved to be involved in salt-induced peptide formation [2]. Despite the plethora of experimental studies concerning Cu^{2+}-polypeptide complexes, theoretical studies are significantly more rare, although molecular modeling and quantum chemistry techniques may provide complementary information, such as data on the structural and exegetic properties of metal-polypeptide complexes [3–25]. Studies have shown that the insertion of β-amino acid residues into peptide chains leads to an increase in their proteolytic stability [26, 27].

Recently, one of us has investigated experimentally the binding of Cu^{2+} to βXaaHisGlyHis, where X=Asp,Ala [28]. Several important observations regarding the formation of complexes at various pH values were presented. However, some issues remained unresolved, e.g., the conformation of deprotonated forms or missing spectroscopic data for some species. Although theoretical modeling of bioinorganic complexes with transition metals might be useful in completing the picture, it is by no means a trivial task. The reasons for this are numerous. Due to the large size of the molecules involved, accurate ab initio methods like coupled cluster formalism [CCSD,CCSD(T)] or CASPT2 cannot be applied. And even if they could, the area of probed conformational space would be very limited. Performing computations of energetic properties with the aid of density functional theory (DFT) is usually feasible for bioinorganic systems involving copper [29, 30] but only a limited number of exchange-correlation functionals have been proved to provide satisfactory results. Turning toward UV-Vis spectra, one finds that most functionals suffer from what is known as self-interaction error. As a result, excitation energies to charge-transfer and Rydberg states are not predicted correctly. Thus, in the field of transition metal chemistry, any computational protocol must be extensively tested against available experimental data before the results can be considered reliable.

The motivation for this study was threefold. First, we aimed to analyze the structural aspects of the binding of Cu^{2+} to polypeptides. Second, we wanted to discuss binding strength together with the analysis of its nature by performing intermolecular interaction decomposition. And a third aim of this study was to compare predictions of

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various exchange-correlation functionals used within the DFT framework for studying Cu^{2+}-containing polypeptides. Although the motivation to undertake this latter subject was purely methodological in nature, such comparisons have become an essential part of contemporary computational chemistry.

Computational methodology

In order to reliably probe the conformational space of the studied complexes, we analyzed several hundred different conformers for all species. In order to find a reasonable compromise between accuracy and computational cost during geometry optimizations, we decided to employ a recently reparametrized NDDO-type semiempirical method called PM6 [31]. As shown recently by Stewart [31], for many properties (including heats of formation, equilibrium geometries or electric-dipole properties) the PM6 method improves upon its predecessor, i.e., the PM3 method. It is also quite successful in determining structures of bioinorganic complexes with transition metals [31]. It has also been shown more recently that this approach can be applied successfully to the modeling of proteins and metaloproteins [32]. Geometry optimization of all conformers, with solvent effects taken into account (see below), was followed by evaluation of a hessian matrix to confirm that stationary points correspond to minima on the potential energy surface (PES). Among all optimized structures, only the lowest-energy conformers for each species are analyzed in the next section. The relative stability of conformers was determined using structures optimized with the PM6 method. In this event, we used several levels of theoretical approximation, including the Hartree-Fock (HF) method, second-order Møller-Plesset (MP2) perturbation theory and the Kohn-Sham formulation of DFT combined with Gaussian basis functions and Hay-Wadt effective core potentials (ECPs) for copper, which incorporate mass-velocity and Darwin relativistic effects [33, 34]. Unless otherwise stated, all computations were performed with inclusion of solvent effects (water). In that event we used a polarizable continuum model (PCM) [35–39]. All the calculations described above were performed within unrestricted formalism using the Gaussian 09 suite of programs [40].

In the present contribution we also made an attempt to analyze the intermolecular interactions between copper ion and the surrounding ligands. In order to compute the total interaction energy, we used a supermolecular approach corrected for what is known as basis set superposition error (BSSE) using the scheme proposed by Boys and Bernardi [41]. To analyze the importance of interaction energy components other than electrostatics (which, in the case of the studied systems, is expected to be main stabilizing factor), we adopted the hybrid variational-perturbational scheme [42–46]. In this approach, the total interaction energy calculated in a supermolecular approach at the MP2 perturbation theory level is partitioned into HF and Coulomb electron correlation interaction energy components:

$$\Delta E^{\mathrm{MP2}} = \Delta E^{\mathrm{HF}} + \varepsilon_{\mathrm{MP}}^{(2)} \tag{1}$$

The HF interaction energy term can be further divided into the Heitler-London interaction energy, which encompasses the electrostatic interactions of unperturbed monomer charge densities as well as the associated exchange repulsion, and the $\Delta E_{\mathrm{del}}^{\mathrm{HF}}$ component comprises the induction and the associated exchange effects [47, 48].

$$\Delta E^{\mathrm{HF}} = \Delta E_{\mathrm{del}}^{\mathrm{HF}} + \Delta E^{\mathrm{HL}} = \Delta E_{\mathrm{del}}^{\mathrm{HF}} + \varepsilon_{\mathrm{el}}^{(10)} + \varepsilon_{\mathrm{ex}}^{\mathrm{HL}}. \tag{2}$$

The second order electron correlation correction term, $\varepsilon_{\mathrm{MP}}^{(2)}$, can be partitioned into the second order dispersion interaction and the correlation corrections to the HF components [44, 45].

$$\varepsilon_{\mathrm{MP}}^{(2)} = \varepsilon_{\mathrm{disp}}^{(20)} + \varepsilon_{\mathrm{el,r}}^{(12)} + \Delta E_{\mathrm{ex-del}}^{(2)}. \tag{3}$$

The $\varepsilon_{\mathrm{el}}^{(10)}$ and $\varepsilon_{\mathrm{disp}}^{(20)}$ contributions are obtained in a standard polarization perturbation theory,[49] whereas the $\varepsilon_{\mathrm{el,r}}^{(12)}$ term is calculated using the formula proposed by Moszynski et al. [50]. In all necessary calculations, the dimer-centered basis set was used consistently and therefore the results are BSSE-free due to the full counterpoise correction [41]. More details about the interaction energy partitioning scheme and the recent implementation adopted in this work can be found elsewhere [51–53]. In the present contribution we compute only $\Delta E_{\mathrm{del}}^{\mathrm{HF}}$, $\varepsilon_{\mathrm{el}}^{(10)}$ and $\varepsilon_{\mathrm{ex}}^{\mathrm{HL}}$.

Results and discussion

In the present contribution, we report an analysis of numerous conformers of βAspHisGlyHis and βAlaHisGlyHis and their deprotonated forms. As mentioned in the previous section, only the lowest energy conformers (confirmed to correspond to minima on the PES) were considered from among initial set comprising almost 1,000 structures. The species analyzed (Fig. 1) were grouped into five categories according to their terminal amino acid (Asp: **A**,**B**; Ala: **C**, **D**, **E**) and the number of dissociated protons (CuL: **B**, **D**; $CuH_{-1}L$: **A**, **C**; $CuH_{-2}L$: **E**). It is important to stress that various protonation states of the investigated species can be observed experimentally as the pH of the aqueous solution changes. Thus, in quantum-chemical calculations, we account for this effect by considering the three most common protonation states.

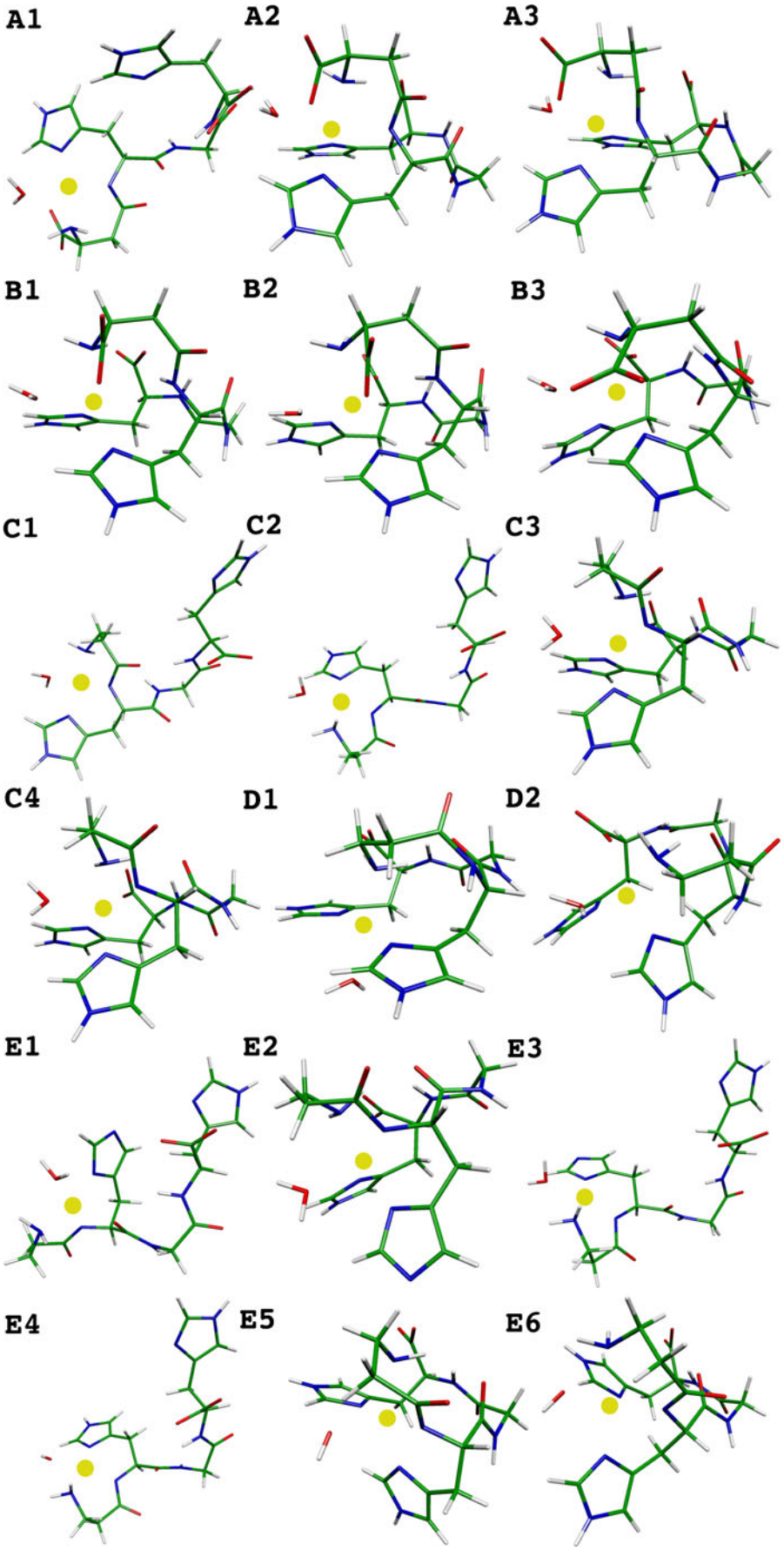


Fig. 1 Structure of the most stable complexes of the species investigated in the present study

Before proceeding to discuss the relative stabilities of the systems under consideration together with their geometrical aspects, we feel that a few words regarding the reliability of applied computational methods are required. Of the plethora of exchange-correlation functionals, only some are suitable for analysis of thermochemical properties or barrier heights. The other issue is that only a few can be used reliably for studies of bioinorganic complexes with transition metals. One of the functionals that preserves a good balance of the description of bonds between transition metals and metal–ligand bonds is the M05 functional proposed by the Minnesota group [54]. The paper presenting the new M05-2X functional also performed an extensive testing of M05 [55]. The former contains double the amount of HF exchange with respect to the latter, namely 56%.

As is well recognized nowadays, problems in describing systems with significant static (nondynamic) electron correlation have their roots in the amount of HF exchange present in the hybrid functional [56]. As pointed out by Cramer and Truhlar, the multi-reference character seems to be much more important for the failure of HF exchange-rich functionals than the presence of transition metal(s) in the system [56]. In general, the performance of M05-2X in determining the properties of transition metal complexes where multi-reference description is needed is much worse than that of M05. This observation, i.e., the difference between the results computed using M05 and M05-2X, might be sometimes used as a rule of thumb to judge if nondynamic correlation effects are important. In the present study we compute total energies in the presence of a water environment using several exchange-correlation functionals including M05, M06 (a redesigned and reoptimized M05 functional that might be considered more accurate) as well as M06-2X and M06-HF. In particular, the latter contains 100% HF exchange. The results of such computations are presented in Table 1. First, let us note that, for the M05 functional, the inclusion of the Hay-Wadt ECP for copper (labeled as HW) leads to an average difference in relative stabilities of 0.62 kcal/mol. As the picture is quite similar for other employed methods, we present only data determined with the use of ECP. Comparing M05 and M06 functionals reveals that it is the charge of system rather than the amino acid (Asp vs Ala) that seems to determine the difference in relative stabilities. Indeed, we note that, for neutral complexes (**B1**-**B3** and **D1**-**D2**), the differences are not significant and the ordering of conformers is the same.

In order to judge the multi-reference character of the systems let us compare how the relative stabilities vary upon increasing the HF exchange (28%→56%→100%). Doubling the amount of the HF exchange from 28% to 56% in most cases reduces the differences in stability between various conformers. An exception is the group of complexes denoted as **C***n*, where the situation is more complicated (although in terms of magnitude the changes are not significant); in this case increasing the amount HF exchange does not lead to reordering of conformers. It is

Table 1 Relative energies including solvent effects for the group of compounds investigated. All values were determined with the 6-31G(d) basis set for molecules and are given in kcal/mol

	B3LYP	B3LYP,HW	M05	M05,HW	M06,HW	M06-2X,HW	M06-HF,HW	MP2,HW	HF,HW
A1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A2	20.63	19.75	19.56	18.75	9.16	4.57	1.54	2.85	24.05
A3	19.66	18.68	18.44	17.52	8.13	3.74	0.59	1.80	22.91
B1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.87
B2	24.01	24.61	24.41	24.37	24.80	19.59	17.31	20.48	23.72
B3	1.22	3.13	1.27	2.86	4.17	2.72	1.77	6.65	0.00
C1	0.47	0.37	1.02	0.00	9.75	10.75	12.00	11.86	0.14
C2	0.28	0.00	1.73	0.55	10.58	11.91	100.00	13.10	0.00
C3	0.00	1.62	0.00	0.18	0.12	0.11	0.12	0.11	14.69
C4	0.03	1.57	0.03	0.12	0.00	0.00	0.00	0.00	14.72
D1	30.34	28.53	28.86	27.47	32.66	35.49	36.56	35.66	33.53
D2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E1	9.34	9.36	6.89	5.87	6.53	5.89	5.87	5.66	02.30
E2	10.77	12.03	7.66	8.05	0.00	0.00	0.00	0.00	18.64
E3	7.56	6.47	5.96	4.59	6.31	8.22	10.77	9.58	0.94
E4	3.51	3.24	5.37	5.10	5.85	7.97	10.49	10.10	0.00
E5	16.25	18.21	16.48	17.45	8.98	6.99	3.32	8.91	21.58
E6	0.00	0.00	0.00	0.00	0.22	2.28	3.28	2.77	0.24

also worth noting that both M06 and M06-2X predict the same most stable conformers in all groups. Likewise, in the large majority of cases, any further increase in the amount HF exchange diminishes the differences in relative stability. The energy difference obtained for the structure denoted as **C2** seems to be an artifact. The spin contamination for this conformer is similar to the values obtained using other functionals. Despite much effort, we did not succeed in locating a lower-energy state. In summary, based on the data presented in Table 1, we may conclude that the results are not sensitive to the amount of exact exchange in the exchange-correlation functional, which might suggest that a single-reference approach is sufficient to describe the studied systems [56]. Hereafter, we shall consider the M06 functional as a reference point for further analysis of the stability of these complexes.

In the following, we discuss the structural aspects of copper coordination by βXaaHisGlyHis peptide in a water environment. The discussion is based on a set of selected geometries of lowest-energy conformers determined using the PM6 method. Considering the size of the molecules studied, it would be a formidable task to probe reliably the potential energy hypersurface using a more accurate method including electron correlation. Thus, to validate the selected set of structures we performed an additional re-optimization of two structures belonging to its smallest subset (denoted as **D**). The differences in geometries optimized using the PM6 and the B3LYP methods [using the 6-31G(d,p) basis set] are presented in Fig. 2 (hydrogens are not shown for the sake of clarity). It should be mentioned that the least-square fit analysis was performed for the three-atom pairs involved in coordination. The relative stabilities obtained (using the M06 method and the 6-31G(d) basis set with the HW pseudopotential for copper) for geometries optimized at the B3LYP/6-31G(d,p) level of theory were 28.58 and 0.0 kcal/mol for **D1** and **D2**, respectively. The energy difference is comparable to that obtained using the PM6 geometry, i.e., 32.66 kcal/mol. In order to unequivocally assess the performance of the PM6 method in geometry prediction, a much more systematic characterization of PES is required.

We included one water molecule in the Cu^{2+} coordination shell in all the investigated complexes. What we observe is that the coordinated water molecule is usually located at longer distances from the metal center in comparison with other ligands (Cu^{2+}–O distance lies within the range 2.03–2.16 Å). In general, the bond distances between Cu^{2+} and all ligands lie in the range of 1.9–2.2 Å, which is in good agreement with experimental values [57]. As far as the $CuH_{-1}L$ form of βAspHisGlyHis is concerned, two main ways of coordination are found. The binding of Cu^{2+} to the peptide in the case of **A2** and **A3** involves the $–NH_{2,\ terminal}$ and $–COO^{-}$ groups of aspartic acid, as well as the nitrogen amide and imidazole ring of first and second histidine. Among the structural motifs observed for **A2** and **A3** there are also three chelate rings (one five-, six- and seven-membered ring). The five-membered ring involves the Cu–carboxyl moiety and the amino group while the six-membered ring is formed by the Cu^{2+}–nitrogen of the imidazole bond and the nitrogen of first amide donor atom. Both structures are also stabilized by a hydrogen bond involving the C-terminal carboxyl group and the N-terminal amino group at a distance of 2.08 Å. In the **A1** structure, coordination of the metal atom by the amino group, the nitrogen atom of the amide group and the imidazole ring of the first histidine is observed. The second imidazole ring is not involved in copper coordination in this structure. Thus, a pentacoordinated $CuH_{-1}L$ structure is found to be more stable than a hexacoordinated one. It follows from Table 1 that the **A1** conformer is a few

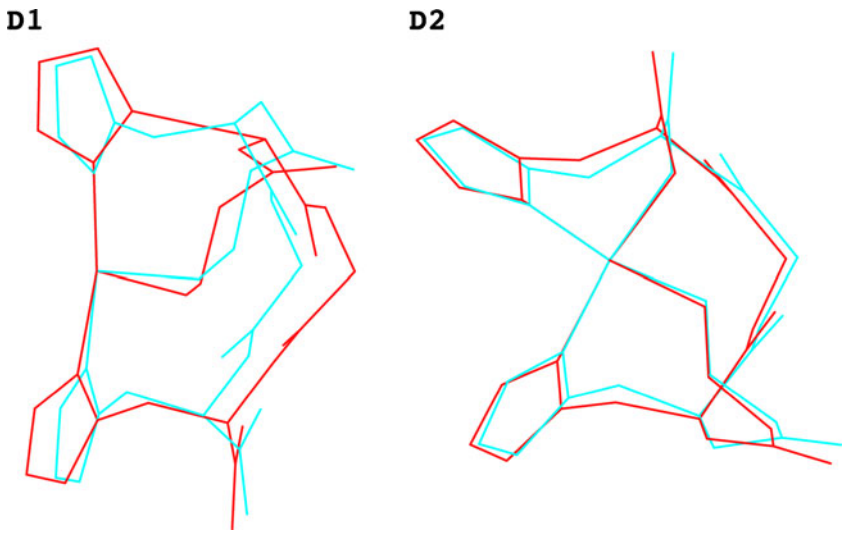
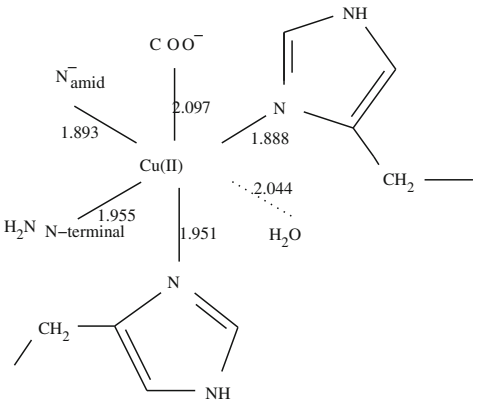
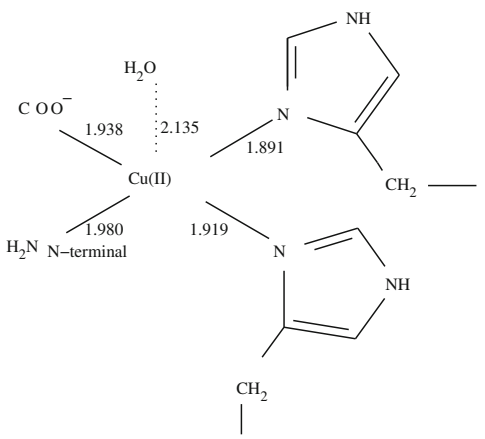


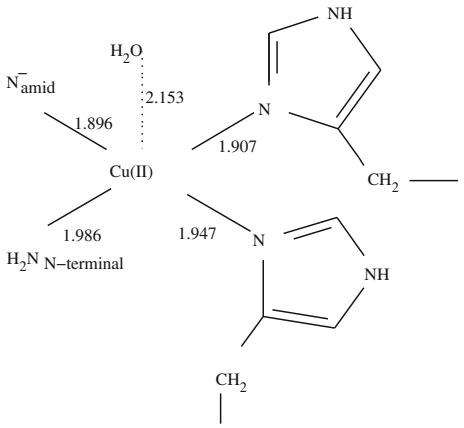
Fig. 2 Differences in structural parameters determined using PM6 (*cyan*) and B3LYP/HW,6-31G(d,p) (*red*) methods



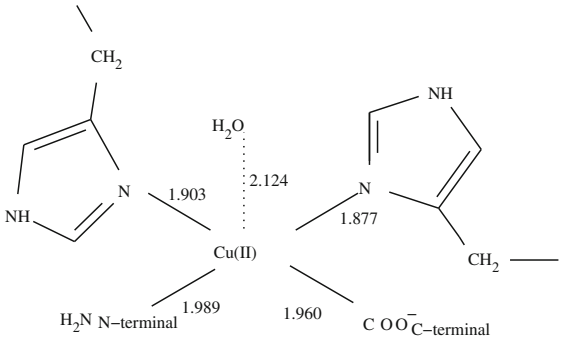
NH_2–β Asp–His–Gly–His–COO^- $CuH_{-1}L$ (**A2, A3**)



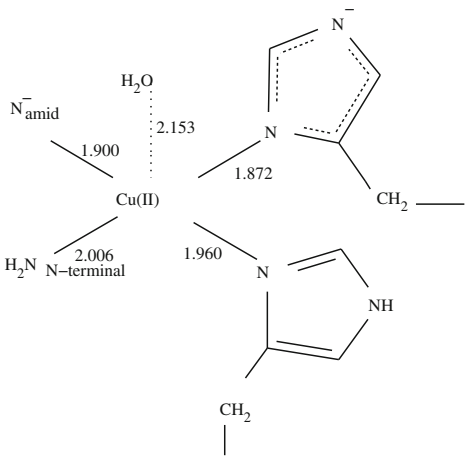
NH_2–β Asp–His–Gly–His–COO^- CuL (**B1**)



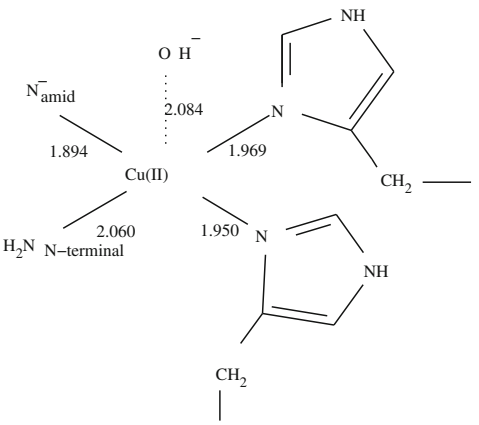
NH_2–β Ala–His–Gly–His–COO^- $CuH_{-1}L$ (**C3, C4**)



NH_2–β Ala–His–Gly–His–COO^- CuL (**D2**)



NH_2–β Ala–His–Gly–His–COO^- $CuH_{-2}L$ (**E2**)



NH_2–β Ala–His–Gly–His–COO^- $CuH_{-2}L$ (**E5**)

Fig. 3 Structural parameters of coordination sites

kcal/mol more stable than **A2** and **A3**. This might be connected with steric effects and the existence of two negatively charged groups (C-terminal carboxyl and N^{-}_{amide}) at a distance of 4 Å, which leads to an unfolded structure. In the peptide with β Ala, the distance is found to be 1 Å longer and the pentacoordinated $CuH_{-1}L$ structure is more stable than the tetracoordinated one. The βAspHis-GlyHis peptide also exists in the CuL form, in which Cu^{2+} is coordinated by the N-terminal amino group, $–COO^{-}$ and the nitrogens of imidazole ring. Moreover, the formation of the five-membered chelate ring between the $–NH_{2,terminal}$ and the carboxyl group is one of the main structural motifs observed in all complexes with Asp. What we also see is that the **B1** structure is stabilized by three hydrogen bonds formed by the hydrogen of the N-terminal NH_2 group and the oxygens of the C-terminal carboxyl group (with distances of 1.93 and 3.12 Å, respectively) and between hydrogen of first amide group and oxygen of the second histidine (1.87 Å). The pentacoordinated complex is the most stable. In the **B2** structure, copper is hexacoordinated by three nitrogen atoms, oxygens from the C-terminal carboxyl group and the side chain carboxyl groups of aspartic acid and water; it is the least stable structure in this set of complexes.

The **B3** complex is about 20 kcal/mol more stable than **B2**, and Cu^{2+} in this structure is bound by the nitrogens of the imidazole rings and amino group and the C-terminal carboxyl group of the peptide. The most stable structures among $CuH_{-1}L$ complexes with β Ala are those denoted as **C3** and **C4** with pentacoordinated copper. **C1** and **C2** are about 10 kcal/mol less stable, and the nitrogen of the second imidazole is not involved in coordination of the copper ion. As far as the βAlaHisGlyHis peptide is concerned, we found only its two stable CuL forms. In the case of the **D1** structure, the 3 N NH_2, $2\times N_{Im}$, O H_2O equatorial coordination is observed. This structure is also stabilized by two hydrogen bonds involving hydrogens of the N-terminal amino group and oxygens belonging to the C-terminal carboxyl group and the carbonyl group of the first histidine. The **D1** complex is about 27 kcal/mol less stable in comparison with **D2**. In the latter structure, we observe that the Cu^{2+} is pentacoordinated, displaying square planar equatorial coordination with three nitrogens and one oxygen belonging to the C-terminal carboxyl group and the axial oxygen from the water molecule. In addition, stabilization also comes from one hydrogen bond (1.933 Å). In the case of all structures belonging to the $CuH_{-2}L$ group, five ligands participate in cooper coordination. The data obtained suggest the existence of two different complexes. The **E1**–**E3** complexes are stabilized mainly by 4N (NH_2, N_{amide}^{-}, N_{Im}, N_{Im}^{-}), O (H_2O) coordination of copper, which results in a square pyramid alignment. There is an experimental evidence for formation of such a structure [28]. The **E4**–**E6** group contains complexes where Cu^{2+} is coordinated by four nitrogens (NH_2, N_{Im}, N_{amide}^{-}) and oxygen (OH^{-}). Moreover, these

Table 2 Basis set superposition error (BSSE)-corrected intermolecular interaction energy (given in kcal/mol) for Cu^{2+}-βXaaHisGlyHis complexes in the gas phase. Unless otherwise indicated, the results were obtained with 6-31G(d) basis set [the values given in parentheses for structures **A1**–**A3** were computed using the 6-311+G(d,p) basis set]. *HW* stands for the Hay-Wadt pseudopotential for copper [33, 34]

	HF/HW	HF	B3LYP/HW	B3LYP	M06/HW	M06	M06-2X/HW	M06	M06-HF/HW	M06-HF
A1	-808.29	-810.58	-868.23	-870.66	-863.95 (−836.11)	-866.86	-861.84	-856.33	-854.33	-861.11
A2	-861.40	-862.79	-913.15	-916.02	-908.88 (−879.36)	-912.83	-912.17	-906.92	-910.70	-916.12
A3	-863.17	-864.63	-914.48	-917.35	-909.80 (−881.10)	-914.34	-913.70	-908.49	-912.37	-917.87
B1	-699.58	-701.32	-756.92	-759.33	-752.17	-756.04	-754.56	-747.89	-749.29	-754.27
B2	-719.47	-721.04	-768.87	-771.01	-763.70	-768.28	-769.87	-764.15	-767.34	-772.50
B3	-698.82	-701.69	-751.03	-755.63	-745.92	-752.19	-749.67	-745.59	-745.91	-753.01
C1	-607.64	-609.22	-708.84	-698.04	-705.53	-691.02	-688.90	-652.04	-653.28	-658.35
C2	-621.80	-580.40	-707.89	-699.96	-704.40	-693.13	-679.03	-669.72	-667.28	-672.68
C3	-712.17	-713.51	-773.22	-775.18	-769.56	-772.88	-767.86	-760.30	-762.01	-766.51
C4	-712.39	-713.69	-773.38	-775.33	-769.81	-773.02	-767.95	-760.48	-762.24	-766.69
D1	-504.16	-438.53	-575.74	-572.74	-571.16	-567.73	-562.61	-553.16	-552.59	-556.47
D2	-565.40	-566.78	-621.01	-622.43	-616.93	-619.84	-617.84	-610.87	-613.37	-618.31
E1	-832.72	-834.77	-890.80	-891.12	-887.45	-887.81	-883.10	-876.15	-874.46	-880.59
E2	-865.59	-867.04	-925.60	-928.15	-922.25	-926.07	-920.09	-912.86	-913.41	-918.12
E3	-788.93	-790.64	-858.11	...	-854.54	-850.96	-844.66	-835.55	-831.74	-837.26
E4	-859.70	-862.09	-912.27	-919.19	-909.54	-915.57	-909.07	-903.88	-900.85	-908.03
E5	-906.84	-908.39	-956.16	-961.17	-951.64	-958.63	-955.76	-950.87	-953.83	-959.24
E6	-858.20	-860.82	-913.56	-919.37	-911.01	-916.18	-909.75	-904.12	-900.89	-908.38

Table 3 Interaction energy components (given in kcal/mol) for selected complexes calculated with the aid of the 6-31G basis set. Values computed using the 3-21G basis set are in parentheses

	$\varepsilon_{el}^{(10)}$	ε_{HL}^{ex}	ΔE^{HL}	ΔE_{del}^{HF}	ΔE^{HF}
B1	-686.03 (−723.54)	209.02 (223.69)	-477.01 (−499.85)	-221.75 (−224.92)	-721.60 (−724.77)
C1	…(−652.68)	…(238.32)	…(−414.36)	…(−210.49)	…(−624.85)
E1	-840.23 (−875.27)	217.41 (233.75)	-622.83 (−641.52)	-234.36 (−214.67)	-857.19 (−856.20)
E5	-888.81 (−925.22)	201.04 (215.53)	-687.77 (−709.69)	-244.78 (−227.37)	-932.54 (−937.07)

latter complexes contain two six-membered chelate rings. Structural data for selected complexes are presented in Fig. 3.

Table 2 lists the results of calculations of intermolecular interaction energies in the gas phase between Cu^{2+} and its surrounding ligands (treated as a whole monomer). The rationale for the neglect of solvent effects (although they are included partially due to the presence of the water molecule in the coordination sphere) is that evaluation of BSSE in the solvent, which is represented as dielectric medium, leads to ambiguity regarding definition of the cavity. One possibility to overcome this difficulty is to evaluate BSSE correction for the complex in the gas phase, and to assume it to be constant in the presence of solvent [58]. However, we have not attempted to use this approach in order to avoid ambiguities in cavity definitions because, in the studied systems, BSSE correction did not exceed 5% of the interaction energy value when using the 6-31G(d) basis set. As seen above, the interaction energies were quite insensitive to the functional employed. On the other hand, the differences are much larger if electron correlation is not taken into account. It is not surprising that the ordering of conformers then differs from that found in Table 1. This is because the interaction energy does not capture the effect of stabilization due to the geometry change in the polypeptide chain. Nevertheless, the values presented in Table 2 might help, although not always, to correlate the binding preferences of Cu^{2+} for complexes of similar conformation with the experimental species distribution. For βAspHisGlyHis and βAlaHisGlyHis, the dominant forms were found to be CuL and $CuH_{-1}L$, respectively. Based on the data in Table 2, one finds that, in the case of βAspHisGlyHis, quantum-chemical calculations predict the CuL form to be less stable than the $CuH_{-1}L$. This disagrees with experimental observations. However, in the case of βAlaHisGlyHis both experiment and theory predict the $CuH_{-1}L$ form to be most stable. The data in Table 2 also confirm another important experimental finding, i.e., species with Asp bind copper more effectively than do species with Ala. The sources of this greater binding ability are due to larger electrostatic interactions [see Table 3 for the results of interaction energy decomposition (Cu^{2+} interacting with ligands in the gas phase)]. In this context, we should mention an attempt to analyze the nature of intermolecular interactions in complexes containing Cu^{2+} recently made by Comba et al. [59]. These latter authors used the KS formulation of DFT to decompose the intermolecular interaction energy into three contributions, namely electrostatic, Pauli repulsive interaction and an electronic stabilization term. As seen in Tables 3 and 4, the electrostatic component is the most important of these, and only 30–50% of its value is cancelled out by associated exchange repulsion. Interestingly, induction effects (present in the delocalization component) are constant and do not depend on the level of deprotonation. The data presented in Table 4 provide us with two important observations. Firstly, the ratio of interaction energy components does not change substantially upon basis set extension. The same is true for the total interaction energy computed using the MP2 method. Secondly, the difference in the HF and the MP2 interaction energies does not exceed 25 kcal/mol. This indicated that, in terms of magnitude, the dispersion interaction is much smaller than the electrostatic term. The reason for presenting the interaction energy components using the 3-21G basis set is that, for some structures, we were not able to converge the dimer wave function to proper solution in larger basis sets. This is because the current implementation of variational-perturbational scheme allows only for the initial guess of orbitals in the form of the Heitler-London wave function. The values of contributions presented in Tables 3 and 4 should be treated with a bit of scepticism as interfragment separations lie in the range 1.9–2.2 Å. For such distances, symmetry adapted

Table 4 Basis set dependence of the interaction energy components (given in kcal/mol) for the complex denoted as **D1**

	$\varepsilon_{el}^{(10)}$	ε_{HL}^{ex}	ΔE^{HL}	ΔE_{del}^{HF}	ΔE^{HF}	ΔE^{MP2}
HF/HW,6-31G	-504.26	217.89	-286.37	-238.04	-524.40	-540.33
HF/HW,6-31G(d)	-433.88	197.64	-236.24	-267.68	-503.93	-527.79
HF/HW,6-311G(d)	-428.35	195.99	-232.36	-270.03	-502.39	-523.83

perturbation theory is expected to be either not quite applicable or to provide only semiquantitative results.

Conclusions

In this study we analyzed structural and energetic aspects of the binding of Cu^{2+} to polypeptides. Numerous lowest energy conformers of βAspHisGlyHis and βAlaHisGlyHis and their deprotonated forms were described. It was found that, in most cases, Cu ion is either hexa- or pentacoordinated. Based on stability analysis, several conformers of similar energy were found for each deprotonated form. In most cases, our computations confirm experimental findings regarding the ability to bind Cu^{2+} at various pH. As a part of a model study, we also made an attempt to critically assess the theoretical methods used to describe energetic properties. In particular, it was shown that the results are not sensitive to the amount of exact exchange in an exchange-correlation functional, which might suggest that a single-reference approach is sufficient to describe the studied systems [56]. However, multiconfigurational wave function- based approaches should be applied in order to unequivocally assess the reliability of the applied protocol.

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