On the Copper(II) Ion Coordination by Prion Protein HGGGW Pentapeptide Model

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The interaction of the octapeptide domain of the prion protein with the transition-metal-ion Cu^{2+} was studied at the DFT level by using the HGGGW pentapeptide as a model to mimic the PHGGGWGQ octarepeat sequence. Ten complexes, in which the metal ion exhibits different coordinations, were considered. Our results indicate that the lowest-energy structure is characterized by a tetracoordinated metal center and that this tendency of the ion to assume the square planar geometry is strong enough to prevent the addition of a further water molecule in its coordination sphere. The role of tryptophan was found to cause a lowering of the system energy due to the stabilizing effect of the electrostatic interaction between the Trp aromatic indole and histidine imidazole rings.

Introduction

The prions (proteinaceous infectious particles, PrP) are aberrant protein forms involved in some of the most feared diseases of the contemporary age.[1−3] They are found mainly in the central nervous system of many mammals and are implicated in a variety of human and animal spongiform encephalopathies, by converting non prion conformers to the prion state.[1,4−9] The normal cellular form of the prion protein (PrP^C), having primarily an α-helical structure (∼40%), converts to an altered isoform (PrP^{Sc}) in which the β-sheet-containing structure increases from 3% in PrP^C content initially to 43%.

As postulated by Cohen and Prusiner,[8] the transmission of disease may be induced by introduction of PrP^{Sc} into the brain, which further catalyzes conversion of the host's normal PrP^C to the abnormal isoform. Other authors suggested that PrP^{Sc} accumulates in the central nervous system in infected individuals and induces neurodegeneration and gliosis, the pathological hallmarks of prion diseases.[10]

PrP^{Sc}, unlike PrP^C, is resistant to digestion with proteinase K (PK) and aggregates to form fibrils.[1,11] Although the studies devoted to the prion diseases are numerous,[1,5,7,11−15] the mechanism by which PrP^{Sc} is formed in vivo from PrP^C is still a debated question.[5,9,15−20]

The amino-terminal region of the human protein, including the 29−124 fragment, is not structured.[21,22] In particular, this region contains four octapeptide repeats of a similar sequence, that is, Pro-His-Gly-Gly-Gly-Trp-Gly-Gln $(PHGGGWGQ)_4$ from residue 60 through 91 one. The octapeptide and its repeats have high affinities for divalent metal ions, in particular, for Cu(II).[23,24] PrP can bind Cu^{2+} inside these regions[23,25,26] and may play a role in copper metabolism,[27−32] but there is no evidence for a behavior characteristic of authentic metal-binding proteins.[33] The role of PHGGGWGQ octapeptide repeats in the prion diseases is confirmed by the fact that, while an increase in repeat number causes spontaneous prion formation, their elimination inhibits propagation. However, this region represents only one of the possible high-affinity binding sites for divalent transition-metal ions among those present within the human prion protein.[33]

Recently, it was shown that two Cu^{2+} ions can bind to a region outside of the octarepeats, between 90 and 126 residues. In this region Cu^{2+} interacts with His111 and His96, promoting the formation of a β-sheet in a region of PrP^C vital for prion propagation.[34]

Studies carried out cooperatively by using CD and NMR spectroscopies on the peptides corresponding to varying copy lengths of the octapeptide motif revealed a stoichiometry of four copper(II) ions per four copies of the octapeptide repeat.[28] Similar findings were obtained by a mass spectrometry investigation.[35]

Full-length PrP binds up cooperatively to four copper ions to the octameric repeat region.[36]

It was shown that a human synthetic peptide (PrP 59−91) corresponding to four octarepeats has the ability to reduce copper and that tryptophan (Trp) is a critical amino acid involved in the oxidation−reduction couple.[9] In fact, a mutant peptide lacking tryptophan displayed only 24% of the wild-type copper reducing activity.[9] When Trp is present in PrP, this becomes a better electron donor, so the amount of observed Cu^+ is higher.[9] Contrary to that, on the basis of previous reduction potential measurements on the systems with and without Trp residue (−0.27 and −0.30 V, respectively), Bonomo et al.[37] rejected the possibility of an easy reduction of the copper center.

A series of Cu^{2+}−peptide complexes obtained by 1-, 2-, and 4-octarepeats and several suboctarepeat peptides was studied using different experimental techniques.[25,33−35,37−43] In particular, the first atomic resolution view of the copper binding site within an octarepeat was presented by Burns et al. [39] In this last work, the X-ray structure of HGGGW in a complex with copper(II) ion reveals equatorial coordination by the histidine imidazole, two deprotonated glycine amides, and a glycine carbonyl, along with an axial water bridging to the Trp indole. Indeed, EPR and CD experiments[38] carried out on the HGGG−Cu^{2+} complex evidenced a different local coordination geometry with respect to the HGGGW−Cu^{2+} species. These studies[38,39]

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emphasize that, at neutral and basic pH, the N_δ atom of the imidazole ring of the histidine side chain as well as amide nitrogens of the backbone are deprotonated.

Most recently, an experimental (vis and EPR, ESI-MS) study on the copper(II) complexes with the Ac-HGGG-NH_2 and Ac-PHGGGWGQ-NH_2 polypeptides suggested a new copper coordination in the octarepeats region.[37] By the ESI-MS spectra, it was found that water molecules are not present in the coordination sphere of the Cu^{2+}, contrary to what was found in the X-ray structure.[39] In particular, the authors emphasized that an eventual apical interaction with water molecule could be present only in the complex with Ac-HGGG-NH_2 species rather than with the Ac-PHGGGWGQ-NH_2 one.[37]

Besides the impressive list of experimental techniques used for determining the structure and the copper-binding properties of the octarepeat region of PrP^C,[25,33,35,37−50] numerous theoretical studies performed at different levels of theory[47,51−55] are available.

In previous quantum mechanical studies, the HGGG and the HG sequences were used as models for Cu(II) binding to the prion protein in the octarepeat region.[51,52]

On the basis of the experimental indication about the importance of tryptophan in the metal-binding mode,[50] in our computations we considered the His-Gly-Gly-Gly-Trp (HGGGW) model of prion including also this residue.

This work was addressed to the characterization of the binding between the Cu^{2+} ion and both HGGGW and HGGGW(H_2O) sequences in order to gain insight into the controversial presence of water molecules in the coordination sphere of the cation. Furthermore, the role of tryptophan on the coordination geometry around the cation was examined.

Computational Methods

All the calculations were performed at the density functional (DF) level of theory using the deMonKS version 2.0[56] and the Gaussian03[57] codes.

Optimization of molecular structures of all complexes was carried out with the deMonKS package employing the PW91PW91[58−60] exchange-correlation functional in connection with the all-electron DZVP[61,62] orbital and A2 auxiliary basis sets for all atoms including the metal ion. Vibrational analysis was performed, at the same level of theory, on the equilibrium geometries of all possible complexes in order to verify the absence of imaginary frequencies.

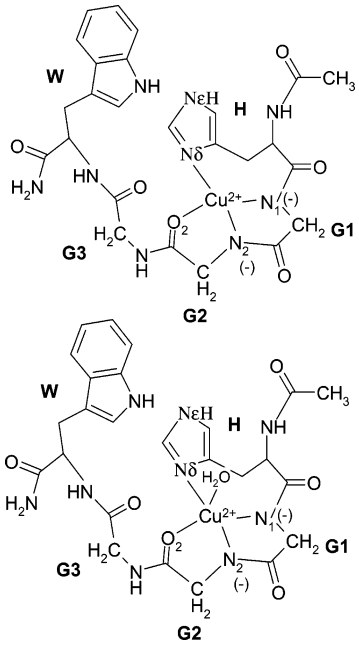
Energy evaluation was made using the hybrid B3LYP[63] functional implemented in the Gaussian03 code.

Structures involving long-range London dispersion forces were re-optimized using an approximate resolution of the identity RI-MP2/SVP[64,65] approach implemented in TURBOMOLE code,[66] in order to validate the DFT results that, as is well-known in these particular cases, must be considered with caution. On the other hand, the RI-MP2/SVP method was previously used successfully to study such type of weak interactions.[67,68]

Solvation energies were obtained as single-point calculations using the self-consistent reaction field polarizable continuum model (SCRF/PCM) procedure[69−71] at both PW91PW91/DZVP and B3LYP/DZVP levels. The surrounding water was modeled using the standard value of 78 for the dielectric constant.

Characterization of the bond type in all the complexes studied was obtained through the NBO procedure, implemented in the Gaussian03 code.[72]

SCHEME 1:



Results and Discussion

The peptide fragment chosen as the working model of the octarepeat region was acetylated at the N-terminal amino acid residue (histidine) and amidated at the tryptophan, giving rise to the Ac-HGGGW-NH_2 system, as in previous works.[34,37,39,51,52]

The labels for the three glycine residues present in the peptide sequence (see Scheme 1) were introduced in order to facilitate the discussion along the text but do not appear in the "HGGGW" general expression.

The presence of a water molecule in the coordination sphere of the copper ion in its complexes with prion protein is an object of a long controversy in the literature. For instance, we mention the recent experimental works of Bonomo et al.[37] and Burns et al.[39] in which this presence is contradicted or suggested, respectively.

For this reason, we have regarded as important the exploration of both hypotheses in order to check if theoretical methods can provide additional contributions to the clarification of the problem.

Four of the five starting structures for DFT optimization were built up similarly to those employed before in an earlier theoretical study,[52] while the fifth one was derived from X-ray coordinates.[38]

Only one coordination water molecule is included in each structure since the other water molecules present in the X-ray one constitute a hydrogen-bond network.

The coordination water molecule was then removed from the five systems obtaining as many dehydrated structures.

Optimized structures without (HGGGW−Cu^{2+}) and with waters (HGGGW(H_2O)−Cu^{2+}) were reported in Figures 1 and 2, respectively (the main geometrical parameters are provided as Supporting Information in Table S1).

For both HGGGW−Cu^{2+} and HGGGW(H_2O)−Cu^{2+} species, the interaction of the ligand with the metal ion always involves the imidazole nitrogen and two amide nitrogen's of the G1 and G2 deprotonated glycine residues. Other attachment sites for the cation can be the carbonylic oxygen atoms of G2 and G3 residues, although the last one is less probable.

Computations were performed with copper in its electronic ground state of doublet that is usually reported as the preferred one in the complexes of this metal ion.[73−75]

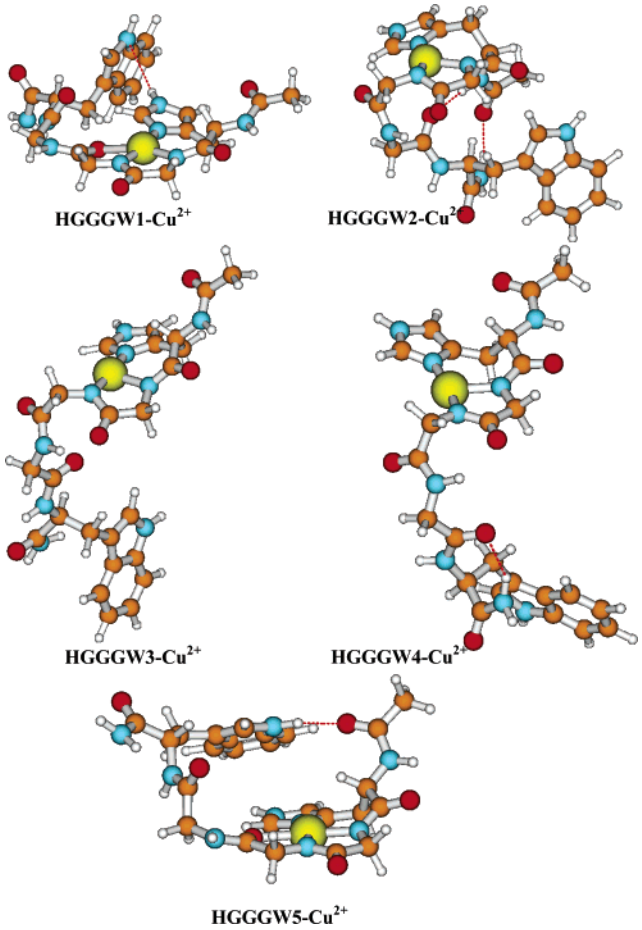


Figure 1. PW91PW91/DZVP-optimized structures of the complexes HGGGW−Cu^{2+}.

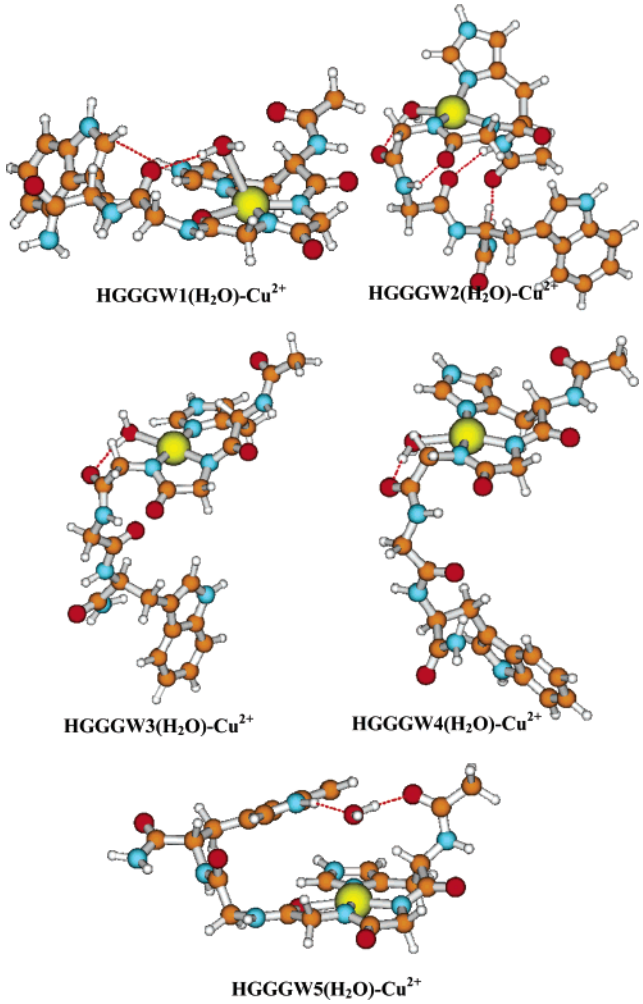


Figure 2. PW91PW91/DZVP-optimized structures of the complexes HGGGW(H_2O)−Cu^{2+}.

HGGGW−Cu^{2+} Complexes. PW91PW91 and B3LYP functionals attribute to the dehydrated HGGGW−Cu^{2+} complexes (see Figure 1) the following orders of stability: 5 > 2 > 3 > 1> 4 and 5 > 2 > 1 > 3 > 4, respectively (see Table 1).

Notwithstanding the differences concerning the energetic gap and the stability inversion between species 1 and 3, both methods indicate the HGGGW5−Cu^{2+} complex as the preferred one.

It is worth noting that for systems involving hydrogen bonds or more, in general, weak bonds, the differences found in the relative energy values obtained by the two PW91PW91 and B3LYP functionals are not surprising, since these computational approaches describe differently these interactions.[76−78] Furthermore, it is necessary to remember that the comparison between these values is made on the basis of data obtained after PW91PW91 optimizations and B3LYP single-point evaluation of energies.

From a conformational point of view, the five equilibrium structures show an orientation of Trp residue totally different. In particular, the species having this residue very far from the metal center (i.e., HGGGW4−Cu^{2+}, HGGGW3−Cu^{2+}, HGGGW2−Cu^{2+}) are not very stable (see Table 1 and Figure 1). The system HGGGW1−Cu^{2+} with the Trp placed perpendicularly to the coordination plane of the cation becomes destabilized because of the presence of repulsive interactions between hydrogen atoms belonging to both Trp and histidine imidazole.

The stability order depends also on the coordination number of the cation. Since it is well-known that copper(II) in its complexes is preferentially tetracoordinated,[73] the species tricoordinated like HGGGW4−Cu^{2+}, HGGGW3−Cu^{2+}, and HGGGW2−Cu^{2+} are consequently the less favored ones. Thus, the most stable species is the HGGGW5−Cu^{2+} in which the metal ion assumes a square planar geometry very similar to that observed in the X-ray structure.[39] Rings of Trp appear to be isoplanar with the coordination plane of Cu^{2+}. This particular orientation of Trp could play a protective shielding role preventing the entrance of further ligands like the water molecules in the metal ion coordination shell, and it introduces a stabilizing effect due to the $\pi-\pi$ interaction between Trp indole and the His imidazole aromatic rings.

A recent investigation on tryptophan binding properties[79] underlines that its indole ring tends to give rise to stacking $\pi-\pi$ interactions with other aromatic rings containing amino acids. This supports our findings, although the DFT results often fail in describing such a type of interaction. This is the reason for which we have redone the optimization of the HGGGW5−Cu^{2+} system, using the RI-MP2/SVP ab-initio method. The structure that we obtained with this last computational tool confirmed the presence of the stacking interaction (distance between Trp indole and His imidazole centroids is 4.058 Å) and, more importantly, appeared to be very similar to that provided by the PW91PW91 density functional calculations.

It is worth noting that the distance between the copper ion and Trp indole ring centroid is very long (5.537 Å) so that, in agreement with Bonomo et al.,[37] we can rule out the involvement of any weak interaction between them.

TABLE 1: Gas-Phase and In-Solvent PW91PW91/DZVP and B3LYP/DZVP Relative Energies (kcal/mol) of the Five Considered Complexes for Both HGGGW−Cu^{2+} and HGGGW(H_2O)−Cu^{2+} Systems[a]

		gas-phase		solvent	
species	μ[b]	PW91PW91	B3LYP	PW91PW91	B3LYP
HGGGW1−Cu^{2+}	14.43	15.1	14.4	3.9	4.9
HGGGW2−Cu^{2+}	12.23	6.1	10.3	7.4	13.2
HGGGW3−Cu^{2+}	11.67	13.2	16.1	8.7	10.3
HGGGW4−Cu^{2+}	13.55	17.9	28.8	9.4	11.5
HGGGW5−Cu^{2+}	9.09	0.0	0.0	0.0	0.0
HGGGW1(H_2O)−Cu^{2+}	13.25	24.2	25.8	12.1	12.3
HGGGW2(H_2O)−Cu^{2+}	12.46	7.5	7.7	6.8	9.9
HGGGW3(H_2O)−Cu^{2+}	10.13	7.9	8.2	0.9	1.1
HGGGW4(H_2O)−Cu^{2+}	13.83	11.5	18.8	1.8	2.4
HGGGW5(H_2O)−Cu^{2+}	7.36	0.0	0.0	0.0	0.0

[a] Gas-phase E_{ZPE} (HGGGW5−Cu^{2+} = −3554.276576 a.u.); solvent E_{SCF}(HGGGW5−Cu^{2+} = −3554.433446 a.u.) at PW91PW91/DZVP level. Gas-phase E_{ZPE} (HGGGW5(H_2O)−Cu^{2+} = −3630.703416 a.u.); solvent E_{SCF}(HGGGW5(H_2O)−Cu^{2+} = −3630.866215 a.u.) at the PW91PW91/DZVP level. Gas-phase E_{ZPE} (HGGGW5−Cu^{2+} = −3554.302478 a.u.); solvent E_{SCF}(HGGGW5−Cu^{2+} = −3554.915882 a.u.) at B3LYP/DZVP level. Gas-phase E_{ZPE}(HGGGW5(H_2O)−Cu^{2+} = −3630.728987 a.u.); solvent E_{SCF}(HGGGW5(H_2O)−Cu^{2+} = −3631.371470 a.u.) at the B3LYP/DZVP level. [b] Dipole moments (μ) are in Debye (D).

Both PW91PW91/DZVP and RI-MP2/SVP Cartesian coordinates of the HGGGW5−Cu^{2+} complex can be found in the Supporting Information (see Table S2).

In the global minimum, NBO analysis indicates that a charge transfer of about 0.8 |e| occurs from ligands to cation. The covalent contribution derives from the overlap between hybrid s(21%)p(79%) orbital of nitrogen of G2 and hybrid s(10%)d-(90%) orbital of the copper ion. Bonds with nitrogens of G1 and His and oxygen of G2 are mainly ionic.

PCM computations in aqueous solution show, for both functionals, a significant reduction of the energy gaps between the various species (see Table 1) and some stability inversion.

PW91PW91 and B3LYP sequences are now 5 > 1 > 2 > 3 > 4 and 5 > 1 > 3 > 4 > 2, respectively. As can be noted, the HGGGW4−Cu^{2+}, HGGGW3−Cu^{2+}, and HGGGW2−Cu^{2+} systems still remain not very stable because of the presence of a tricoordinated cation. For this reason, it is not interesting to try a rationalization of their new position in the stability trend. On the contrary, the species HGGGW1−Cu^{2+} gains both stability and relevance since it now lies at only 3.9 and 4.9 kcal/mol at PW91PW91 and B3LYP levels, respectively, with respect to the global HGGGW5−Cu^{2+} minimum.

A glance at the dipole moment values (μ in Table 1) of these systems in the gas phase reveals the reason for the energy recovery of the complex HGGGW1−Cu^{2+} with respect to HGGGW5−Cu^{2+}. In fact, this species owes its high solvation energy to its high dipole moment. However, it is worth noting that the solvation is not sufficient to balance the intrinsic instability of this system, thus the HGGGW5−Cu^{2+} complex continues to be the most stable one despite its lowest μ-value.

HGGGW(H_2O)−Cu^{2+}. The optimization of the monohydrated systems yielded the five HGGGW(H_2O)−Cu^{2+} structures depicted in Figure 2.

The stability of these complexes follows the order 5 > 2 ≈ 3 > 4 > 1 at both levels of theory.

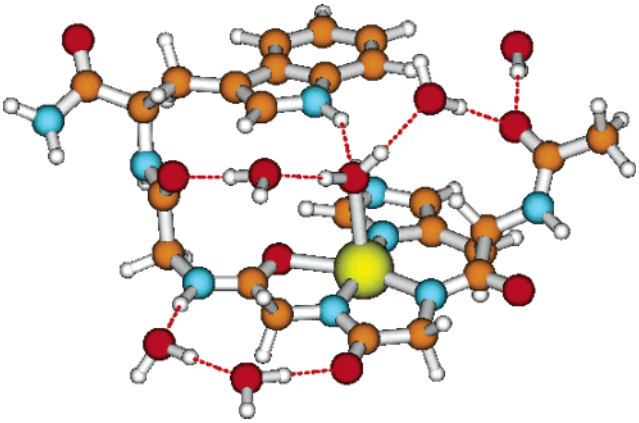


Figure 3. PW91PW91/DZVP-optimized geometry for the X-ray crystallographic structure.

As can be noted from Table 1 that system 1 becomes the least stable one. This fact confirms that Cu^{2+} is unlikely to adopt the pentacoordination, caused by the entry of the new ligand.

Structures 2−4 present around the metal center a slightly distorted square planar geometry, but the Trp residue remains very far from the coordination plane as in analogous cases without water. All HGGGW2(H_2O)−Cu^{2+}, HGGGW3(H_2O)−Cu^{2+}, and HGGGW4(H_2O)−Cu^{2+} complexes show the water molecule on the equatorial plane, and the distance Cu^{2+}−O_w is 2.143, 2.278, and 2.267 Å, respectively.

Species 5 is again the most stable one. The equilibrium geometry is very similar to that obtained for the same complex lacking in water. RI-MP2/SVP computations support also in this case the presence of a stacked conformation (see Table S2 of Supporting Information for Cartesian coordinates). The distance between Trp indole and His imidazole centroids is 4.245 Å, while the distance between Cu(II) and the Trp indole ring is 5.375 Å.

The water molecule does not enter into the coordination sphere of the copper ion, but it is held by two hydrogen bonds to the Trp and to the carbonyl of the group used to simulate the proline residue.

This result is in contrast to the X-ray structure in which a water molecule appears to be linked to the copper ion, but it agrees with the observation of Bonomo et al.[37] concerning the absence of a water molecule in the apical position. The absence of a direct bond with the copper ion is attributed to the presence of Trp residue because the complex that the shorter HGGG fragment forms with Cu^{2+} shows, instead, the water coordinated on the metal center.[37]

In order to verify if the absence of the water−copper ion bond in the HGGGW(H_2O)−Cu^{2+} system is not an effect of the used model, we have done an optimization to the X-ray structure including all the entrapped water molecules. The result of the optimization is illustrated in Figure 3. The similarity to the experimental structure is noticeable. In particular, the geometry obtained theoretically shows that now the water molecule in the apical position is linked (2.475 Å) to the copper ion. The diversity between this structure and that examined before consists only in the different number of water molecules. Therefore, we can hypothesize that the coordination in the apical position of this ligand by Cu^{2+} is possible only if a hydrogen-bond network between water molecules, extending from the backbone carbonyl preceding the histidine to the carbonyl of G3, is present.

In other words, the copper ion prefers the square planar geometry and will tend to assume it both in the gas phase and in solution.

On the other hand, it is well-known that copper dication complexes present the Jahn−Teller effect that consists in an elongation of the bonds that the ion establishes with the apical ligands so that they can be easily lost and their experimental detection becomes difficult.

As in the previous complex without water molecule, NBO analysis suggests that the interaction between the ligand and the cation in HGGGW5(H_2O)−Cu^{2+} is essentially ionic with a slight covalent character deriving from the overlap of nitrogen of G2 and copper ion orbitals.

In aqueous solution the stability order of the complexes changes with respect to that obtained in the gas phase, but both functionals propose the same trend: $5 \geq 3 > 4 > 2 > 1$. Energetic gaps between the various species appear to be sensibly reduced, especially for the complexes having high dipole moment values (see Table 1). In this situation, three systems become almost isoenergetic, thus we cannot exclude their probable existence in solution.

However, in the limits of the theoretical approach, the HGGGW5(H_2O)−Cu^{2+} continues to be the preferred structure.

We think that other solvation methods should be applied to confirm these results. In fact, no survey has been available until now to decide if the PCM approach is quite reliable to describe bulk solvent effects in peptides without the addition of a large number of explicit water molecules. Unfortunately, the resulting size of the systems cannot be treated at a full quantum mechanical level.

Conclusions

The aim of the present work was to provide a detailed analysis of the gas-phase and in-solution binding between the Cu^{2+} cation and the simplest model of the octarepeat domain present in the N-terminal region of the prion protein. Computations were performed in the framework of density functional theory. The following conclusions can be drawn.

- The copper ion interacts with the HGGGW segment, giving rise to different stable complexes.
- The stability order of the dehydrated systems is determined by both the coordination number of the copper ion and the tryptophan residue position. In particular, the global minimum is characterized by the most usual square planar geometry of the copper complexes and by an orientation of Trp that yields an additional contribution to the stabilization energy arising from the electrostatic interaction between its aromatic ring and that of histidine imidazole.
- Results concerning the monohydrated complexes confirmed, limited to the global minimum, the low tendency of the copper(II) ion to assume a pentacoordination. We have ascertained that a direct bond between the metal ion and a water molecule playing the role of fifth ligand is consistent only with the presence of additional water molecules arranged in a hydrogen-bond network. This is more usual in condensed phases.
- The aqueous environment does not significantly change the stability order of the complexes with respect to the gas phase, but the reduction of the energetic gaps between the various hydrated structures suggests the probable coexistence of three species.
- The metal ion−ligand interaction was found to have an electrostatic nature with most of the coordinating groups. The charge-transfer revealed by natural bond orbital analysis is justified by the covalent character of the bond between the copper ion and nitrogen of the glycine (G2) residue.

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Supporting Information Available: Main optimized geometrical parameters (distances) of the five structures for both HGGGW−Cu^{2+} and HGGGW(H_2O)−Cu^{2+} systems. This material is available free of charge via the Internet at http://pubs.acs.org.

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