Articles

Interactions Study between the Copper II Ion and Constitutive Elements of Chitosan Structure by DFT Calculation

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Molecular modeling is particularly useful to understand interactions between various kinds of molecules and ions. This study is aimed at studying the interactions between one Cu^{2+} ion and one or several glucosamine residues. The geometries and the interaction energies of all of the complexes involving all of the dimers obtained from glucosamine and *N*-acetylglucosamine were computed by means of density functional theory (DFT) methods. In a first step, for the two dimers A−A and A−B (A for glucosamine and B for *N*-acetyl glucosamine), a starting geometry was built, and the energies were calculated using a rigid rotation of 30° intervals for each of the dihedral angles (Φ and Ψ) of the glycosidic bond, spanning the whole angular range. These calculations allowed us to retrieve the minimal energy conformation and investigate all possible conformations. The results were compared to some experimental data. In a second step, we investigated the interactions of Cu^{2+} with the different possible coordination sites of A. For all complexes considered, the Cu^{2+} site was completed with H_2O and/or OH^- ligands to have a global neutral charge. The calculations confirmed that the most stable interactions involved the free amino site in a "pending complex". Another pending form was possible considering the participation of the heterocyclic O site, but the latter was less favored. On the other hand, we also showed that glucosamine could not act as a bidentate ligand and that *N*-acetyl glucosamine was not coordinating with Cu^{2+}. Finally, our results evidenced a cooperative fixation of Cu^{2+} ions when considering the complexation of two successive metal ions on the two consecutive glucosamine residues of the dimer A−A.

Introduction

Interactions between chitin, chitosan, or their derivatives and metal ions are of great interest and are particularly important in the fields of bioorganic chemistry[1] or depollution.[2] D-Glucosamine and small oligomers of D-glucosamine and *N*-acetyl-D-glucosamine also form stable complexes. The complexing behavior of these amino-sugars (monomers, oligomers or polymers) is still not fully understood.[3] Although it is generally accepted that the free amino group is mainly involved in the coordination with metal ions, conflicting conclusions based on experimental results concerned the structure of the formed complexes, especially the participation or not of hydroxyl groups in the metal coordination, and the possibility to form chelates. On the other hand, the protected amino group of *N*-acetyl D-glucosamine would have a negligible tendency to coordinate.[1,4]

To clearly answer the questions about the active sites of complexation and the detailed structure of complexes formed with metal ions, we planed a theoretical approach of the problem. Thus, we used quantum ab initio[5,6] and DFT methods.[7] For the first time, we focused our investigations on the interactions between the single monomers: D-glucosamine or *N*-acetyl D-glucosamine and Cu(II) ions. Then, we computed the interactions of the different corresponding disaccharides with these same ions. The ultimate objective was to refine the interactions between the polymer chains and Cu^{2+}.

The aim of the present work was then to report on the results regarding the following: a preliminary work about the theoretical conformational study of the monomers then, of the four derived disaccharides; calculations carried out to locate favorable binding sites for Cu^{2+} on the glucosamine and *N*-acetyl-D-glucosamine monomers to form stable neutral squared plan complexes, in a weakly alkaline moiety; calculations of the interaction energy between a glucosamine dimer with one or two Cu^{2+} observed in stable neutral squared plan complexes and in a weakly alkaline moiety.

Results and Discussions

For the whole work, the glucosamine monomer or residue was referred to A and to B for the *N*-acetyl glucosamine monomer or residue. Heavy atoms of the two monomers were numbered as shown on Figure 1.

The different carbons of the two residues were numbered according to the carbohydrate nomenclature.[8]

1. Energy Surfaces for Dimers. In a preliminary work, we studied a model of the A and B residues, and the structures were geometrically optimized using the quantum chemistry

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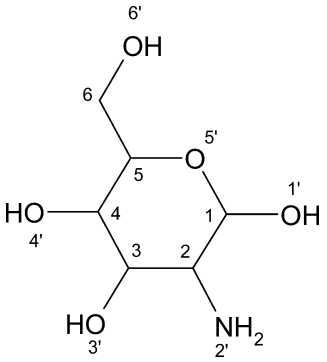
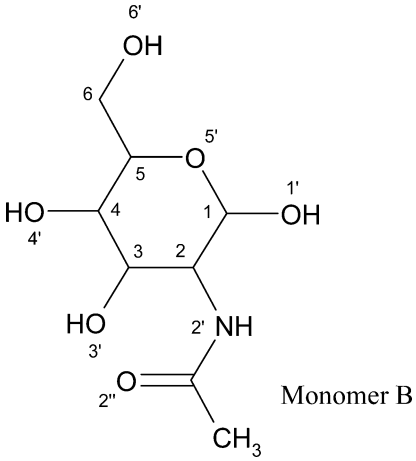


Monomer A



Monomer B

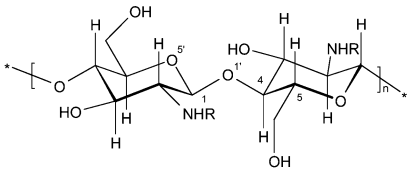
Figure 1. Numbering of the heavy atoms for monomers A and B.

Hartree−Fock (HF) method[5,6] with a 3-21G* atomic basis set (HF/3-21G*).[7] For both A and B structures, all of the computed angles, dihedral angles, and bond lengths were compared to the experimental structures solved by X-ray diffraction.[5] The comparison showed that there was no significant difference between the computed structures and those deduced from experimental results, thus reinforcing the agreement between the two kinds of approaches.

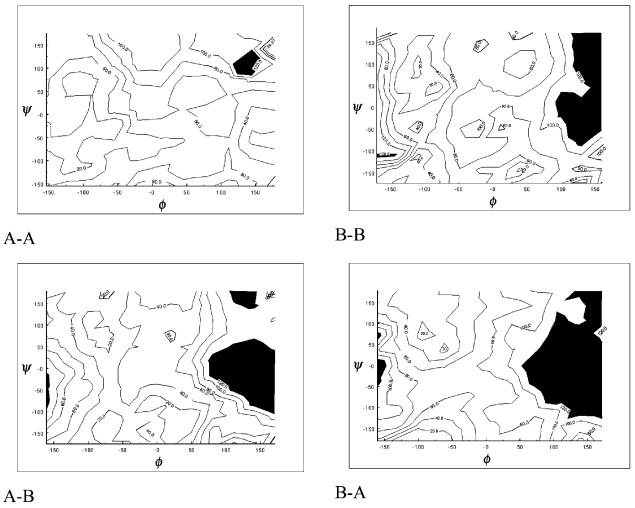
The HF method is a quantum chemistry method using the ab initio HF algorithm. The 3-21G* atomic basis set[9] was used to fully relax the structure and the 6-31G** basis set[10] for some single point calculations. Single point calculations were performed on the fully optimized structure to measure the energy of the molecule or of complexes and to have a fine description of the different molecular orbitals. These procedure were only applied to accurate the energies of extrema in conformational maps. The use of the 3-21G* set allowed a good description of the polarized bonds and also a reliable description of hydrogen bonding. The 6-31G** set[10] was necessary to have a fine description of these weak interactions. The use of these HF methods is in fact a good compromise between the time of calculation and the description of weak interactions such as hydrogen bonding, for organic compounds.[11]

The main objective of this study was to investigate the structure of complexes formed between chitosan chains and ions of heavy metals such as Cu^{2+}. Chitosan chain is relatively rigid, and its main degrees of freedom are due to the two dihedral angles (Φ and Ψ) of the glycosidic bond between two monomer units.

To obtain a reliable model of the chitosan chain, all the conformations allowed between two residues must be known. Thus, to investigate this point, a conformational scan of the two



The dihedral angle (5',1,1',4), (Φ) is reported along the X axis and the dihedral angle (1, 1', 4, 5), (Ψ) is reported on the Y axis.
For the monomer A: R=H ; for the monomer B: R = $COCH_3$.



A-A B-B

A-B B-A

Figure 2. Iso-energy contour maps for the four possible dimers (A−A, B−B, A−B, and B−A). Energetic iso-contour levels are drawn. The global minimum is set to zero.

dihedral angles of the A−A, B−B, A−B, and B−A pairs of residues was computed. For each dimer, each dihedral angle was increased stepwise of 30°. For each step, the energy of the whole structure (except the 2 angles, which were frozen) was optimized (minimized) and calculated with HF/3-21G* methods. A 3D surface was obtained: the values of energy were reported on the *Z* axis and the two dihedral angles on X and Y axes. From this 3D surface, an iso-energy contour map could be plotted. The maps are given for the 4 dimers A−A, B−B, A−B, and B−A on Figure 2.

Whatever the case, the magnitude of the variation of energy was high and over 160 kJ·mol^{-1}. Nevertheless, at room temperature, only areas with less than 20 kJ·mol^{-1} from the global minimum were accessible.[12] An analysis of the four plots of Figure 2 reveals that in all cases, only a few conformations were accessible and that the glycosidic linkage is highly stressed. This result is in good agreement with the well-known stiffness of the $\beta(1\rightarrow4)$ linked polysaccharides.[13]

For the four dimers, the variation of the dihedral angle Φ seems to be weak. Indeed, the variation of this angle is very limited, with wells at about +50° and −100°. The variation of the Ψ angle is more important but for the four cases, there are energy wells roughly centered at −90° and +90°. In the latter case, the structure confirms that each monomer is inverted regarding the previous one. In the case of A−B, B−B, and B−A, this conformation allows the formation of several hydrogen bonds between the *N*-acetamide function and hydroxyl groups of the other monomers. A comparison between B−A and A−B scans reveals that the positions of the different energy wells are almost the same. However, for A−B, the wells are sharper than with B−A. The analysis of the A−A energy surface shows that the area located between 0 and 20 kJ is larger than in all of the other cases. There is a narrow valley allowing the Ψ angle to vary from −90° to +90°, the Φ angle remaining almost close to −100°. This analysis agrees with the structure

proposed from X-ray diffraction.[5] It also suggests that a polymer chain, only constituted with A monomers, would be more flexible than with only B monomers, in agreement with results obtained for solutions of chitosan at various degrees of acetylation.[13]

2. Theoritical Study of Complexes between One Cu^{2+} Ion and Ligands of A Monomer Type. In the second part of this work, we investigated the interactions between one Cu^{2+} ion and one or two A ligands.

2.1. The Models. 2.1.1. Complexes Formed between One Cu^{2+} Ion and One Monomer A. The different oxygen and nitrogen coordination sites of A (D-glucosamine) were successively considered. For each possible heteroatomic binding site on ligand A, a model was built. Thus, from a previous fully optimized A monomer (see part 1), one Cu^{2+} ion was displayed in the vicinity of the chosen heteroatom. Considering a weakly alkaline pH condition as optimal for the formation of complexes between chitosan and copper II ions[1] and according to the well-known square plane configuration of Cu^{2+} complexes, a convenient number of H_2O and OH^- ligands were displayed to ensure the neutrality of the complex. The different investigated complexes $[Cu(OH)_2(H_2O)A_i]$ were noted shortly $[(A_i)]$ with "*i*", the index referred to the atoms numbering of Figure 1; thus, $i = 1'$, $2'$,$3'$, $5'$, and $6'$.

To build reliable conformations, we needed an approximation of the bond length between Cu^{2+} and the different atom sites of the ligands. Then, the simplest models $[Cu(OH)_2(H_2O)(CH_3OH)]$ and $[Cu(OH)_2(H_2O)(CH_3NH_2)]$ were used to approximate the bond lengths. They were built and optimized using the computing method described below. The optimized distance between the Cu^{2+} ion and the oxygen of an alcohol function was found 2.3 Å; the optimized distance between the Cu^{2+} ion and the nitrogen of the amine function was found 2.1 Å. For both of these complex models, the optimized distances with the O of the OH^- or of H_2O were found 1.95 Å. Therefore, and similarly, for each following investigated chitosan complex, the Cu^{2+} ion was initially built with a distance from a heteroatom O or N of the glucosamine equal to 2.3 Å. H_2O or hydroxyl ligands were initially displayed with a distance between the O and copper ion of 1.95 Å.

2.1.2. Complexes Formed between One Cu^{2+} Ion and Two A Monodentate Ligands. With the same investigation procedure, we built different tetracoordinated neutral complexes formed between one Cu^{2+} ion, two A monomer ligands and two OH^- ligands in a weakly alkaline moiety. The different oxygen and nitrogen coordination sites of each A were successively considered. Thus, the considered models were $[Cu(OH)_2(A_i)(A_j)]$, shortly noted $[(A_i)(A_j)]$ according to the numbering shown on Figure 1.

2.1.3. Complexes Formed between One Cu^{2+} Ion and One Bidentate A Monomer. With a same investigation procedure, we also built different tetracoordinated neutral complexes formed between one Cu^{2+} ion, one A monomer bidentate ligand, and two OH^- ligands in a weakly alkaline moiety. Two coordination sites of A (indexed *i* and *j*) were both considered, and then, one A monomer was behaving as a bidentate ligand. Thus, the considered models were $[Cu(OH)_2(A_{ij})]$ shortly noted $[(A_{ij})]$, according to the numbering shown on Figure 1.

2.2. Optimizing Method. From each initial built complex conformation, an optimizing procedure was started using DFT from the ADF software.[14,15,16] The generalized gradient approximation (GGA) was set with the Becke gradient correction methods associated with the Perdew correlation term.[17] The different atomic orbitals of the models were described with the double zeta atomic basis set (DZ).[18] This method of calculation allowed a reliable description of the dative bond and weak interactions such as hydrogen bonding.[19]

2.3. Results and Discussion. The energy of each optimized complex model $[(A_i)]$, $[(A_i)(A_j)]$, $[(A_{ij})]$ was computed. Then for each optimized complex structure of the A oligomer, the energy of interaction (ΔE) between the aqueous copper II ion and the A ligand was calculated corresponding to energies of the following chemical reactions:

$$[Cu(OH)_2(H_2O)_2] + A \xrightarrow{\Delta E} [Cu(OH)_2(H_2O)A_i] + H_2O$$

with a monodentate ligand, or

$$[Cu(OH)_2(H_2O)_2] + A \xrightarrow{\Delta E} [Cu(OH)_2A_{ij}] + 2H_2O$$

with a bidentate ligand.

For this calculation, we had to evaluate the energy of the aqueous copper II ion ($[Cu(OH)_2(H_2O)_2]$). The latter was built and energetically optimized by the method described above. The optimized distance between the Cu^{2+} and the oxygen of the OH^- ligand was found to be about 1.94 Å. The optimized distance between the Cu^{2+} and the oxygen of the water ligand was found at about 2.08 Å.

ΔE calculations were performed using the basis set superposition error (BSSE).[20] The lower the interaction energy ΔE, the more favored the complex was. The interaction energies are reported in Table 1. The analysis of the energies ΔE, in this table, reveals some interesting information:

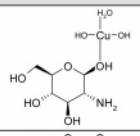
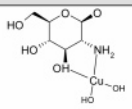
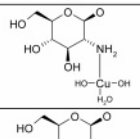
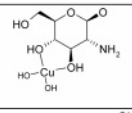
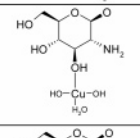
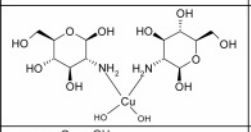
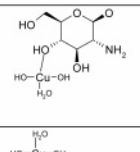
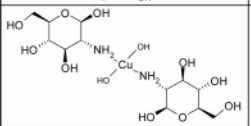
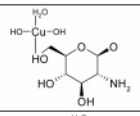
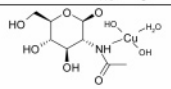
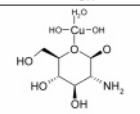
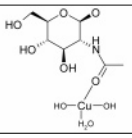
(1) For the pending complexes formed with one monodentate monomer and one copper II ion, the most stable interaction occurs between the nitrogen atom of the amine group and the copper ion (complex noted $[(A_{2'})]$). An interaction with the oxygen inserted in the sugar ring (complex $[(A_{5'})]$) is also possible but less stable of about 7 $kJ \cdot mol^{-1}$. The other complexes formed with the other oxygen sites are energetically disfavored.

(2) All of the bidentate complexes such as $[(A_{2',3'})]$ and $[(A_{3',4'})]$ exhibit very weak interaction energies and then, cannot be formed.

(3) The most stable complex involves two amine groups, each one belonging to a different A monomer: cis$[(A_{2'})(A_{2'})]$. The trans conformation of this complex is less stable as its energy is 12.9 $kJ \cdot mol^{-1}$ higher. A close analysis of the two conformations reveals that, for the trans conformation, the square plan is twisted by a hydrogen bond between a hydrogen atom of the amine function and the oxygen of one hydroxyl ligand. This H bond destabilizes the dative bond between the nitrogen and Cu^{2+}. The angle defined by N, Cu^{2+}, and O of the hydroxyl ligand was found to be 82.1° instead of 90°. On the other hand, the nitrogen electronic lone pair was not appropriately oriented to induce an optimal combination with the orbital of the copper II ion to form a dative bond.

Furthermore, the geometry analysis of the optimized complexes revealed that the distance between Cu^{2+} and oxygen atoms weakly varied (from 2.08 in the complex $[(A_{6'})]$ to 2.11 Å in the complexes $[(A_{2',3'})]$ and $[(A_{3'})]$). For the most stable complex cis$[(A_{2'})(A_{2'})]$, the distances between Cu^{2+} and the two N atoms were shorter by about 0.05 Å. In all complexes, the lengths between Cu^{2+} and OH^- ligands were close to 1.89 Å, although with H_2O ligands they were of 2.05 Å. This bond contraction effect could be attributed to the contribution of the electrostatic energy (OH^- is charged and H_2O neutral).

Table 1. Energies (ΔE) Calculated for the Different Sites of Coordination in the Case of Complexes with One Monomer as a Monodentate Ligand [(A_i)] or [(B_j)], One Monomer [($A_{i,j}$)] as a Bidentate Ligand and Two Monomers A (Each One as a Monodentate Ligand) (cis or trans) for One Copper Ion (cis or trans [(A_i)(A_j)])[a]

Structure	Complex name	Energy (ΔE) (kJ.mol^{-1})	Structure	Complex name	Energy (ΔE) (kJ.mol^{-1})
	[($A_{1'}$)]	-1.87		[($A_{2',3'}$)]	+26.9
	**[($A_{2'}$)]**	**-75.6**		[($A_{3',4'}$)]	+90.0
	[($A_{3'}$)]	-48.4		**cis[($A_{2'}$)($A_{2'}$)]**	-78.4
	[($A_{4'}$)]	-35.6		trans[($A_{2'}$)($A_{2'}$)]	-65.5
	[($A_{6'}$)]	-7.49		**[($B_{2'}$)]**	-5.31
	**[($A_{5'}$)]**	**-68.9**		[($B_{2''}$)]	-51.61

[a] Each index corresponds to the atom numbering shown in Figure 1. For the B ligands, only the amide sites were considered.

The calculation of molecular orbitals showed that for the most stable pending complex corresponding to [($A_{2'}$)], with only one Cu^{2+}, the amino-nitrogen and the oxygen atoms of the three other ligands were involved in the highest occupied molecular orbital (HOMO; Figure 3). (The interaction involves the d orbitals of the Cu^{2+} ion and the p orbitals of the N and O atoms.) The HOMO of [($A_{2'}$)] was strongly located on these five coordinated atoms.

For [($A_{5'}$)], the other possible pending complex (less stable), the HOMO was delocalized on almost the whole molecule. Cu^{2+} and the four coordinated atoms were less involved than some others. This could explain why the [($A_{5'}$)] complex was less favorable than [($A_{2'}$)].

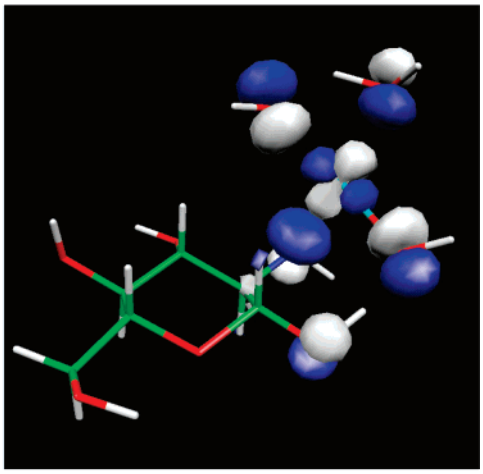
Our results can be confronted with those obtained experimentally by circular dichroism on the monomer glucosamine.[21] In the latter work, the presence of four major complexes was clearly demonstrated, especially in the range of wavelengths located within 500−700 nm. Unfortunately, this technique could not allow a precise determination of the geometry of the complexes. Nevertheless, additional information obtained from these experiments was that one of the complexes formed with the monomer should be the same as the only one formed with the polymer. This complex was proposed to correspond to the pending form [($A_{2'}$)] in Table 1. Another set of structures was proposed from EPR measurements.[4] In this work, five complexes were identified. The proposed structures corresponded to the pending complex mentioned above and derivatives of the complex trans[($A_{2'}$)($A_{2'}$)] in Table 1. Nevertheless, the structures they proposed in the latter case involved either OH groups near the amino groups of each monomer or their deprotonated forms and even a structure with five ligands. From our calculations, most of these last structures cannot be considered as likely to be formed.

3. Theoritical Study of Complexes Formed between One Cu^{2+} Ion and B Monomers. The same investigation procedure as described in part 2 was processed to built different tetracoordinated neutral complexes formed between one Cu^{2+} ion, one B monomer ligand, and two OH^- ligands, in a weakly alkaline moiety. The different investigated complexes, [$Cu(OH)_2(H_2O)B_i$], were noted shortly [(B_i)], according to the numbering shown on Figure 1.

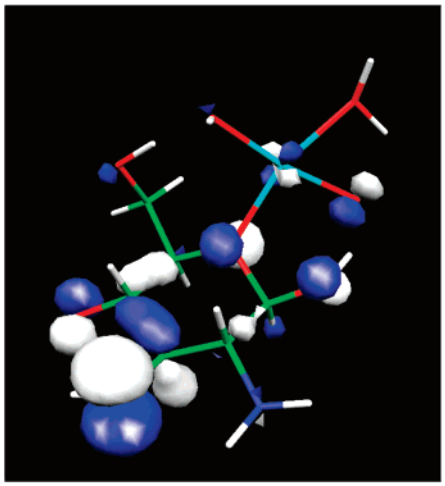
The same computing method as described in part 2 was used to optimize the initially built conformations. For each optimized [(B_i)] complex structure, the energy of interaction ΔE between the copper II ion and the B ligand was calculated. Results are reported in Table 1.

The high interaction energies ΔE between one copper II ion and the N or O binding sites of the amide group (complexes noted [($B_{2'}$)] and [($B_{2''}$)], respectively) agree with the nonexistence of these complexes. For the complexes formed between one copper II ion and the different oxygen binding sites of OH groups of a B monomer (complexes [($B_{3'}$)] and [($B_{5'}$)]), we found the same interaction energies as those for similar complexes obtained with the A monomer. As a conclusion, the difference in behavior between the two kinds of monomers, A or B, comes from the difference between a N binding site of a NH_2 and that of a *N*-acetamide group. The B complex exhibits an energy difference higher than that of A complexes close to 70 kJ·mol^{-1}. Consequently, these results confirm the absence of any strong interaction between Cu^{2+} and B monomers.

4. Theoritical Study of Complexes Formed with One Chelating Dimer (A−A) and Cu^{2+} Ions. We also considered possible interactions between dimers and Cu^{2+}. Thanks to the same theoretical method processed for the above studies, the stability of several complexes was investigated. Since the conclusion of section 3 definitely showed the absence of



$A_{2'}$



$A_{5'}$

Figure 3. HOMO molecular orbitals for the most stable structures corresponding to pending complexes between copper II ions and one monomer A_i.

complexes between Cu^{2+} ions and B monomers, in this part, only neutral complexes between A−A dimers and a copper II ion were considered, in a weakly alkaline moiety.

Starting from the study of the dihedral scan on dimers (part 1), we built a complex between one Cu^{2+}, one A−A chelating dimer (in its more stable conformation), and two OH^-. The different combinations of two heteroatom binding sites of one chelating A−A dimer were considered, involving complexes $[Cu(OH)_2(A_i-A_j)]$, shortly noted $[(A_i-A_j)]$. Then, because some combinations *i*,*j* were geometrically impossible, only five models were constructed: $[(A_{2'}-A_{6'})]$, $[(A_{2'}-A_{3'})]$, $[(A_{6'}-A_{6'})]$, $[(A_{2'}-A_{2'})]$, and $[(A_{2'}-A_{5'})]$.

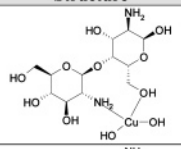
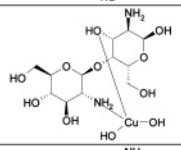
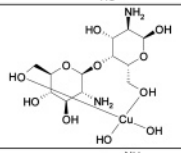
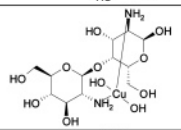
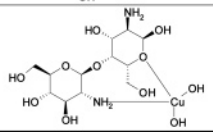
The computed models were verified to avoid the boatlike conformation for the different monomers. For each model, the geometry was energetically optimized. Then, the reaction energies ΔE of

$$[Cu(OH)_2(H_2O)_2] + A-A \xrightarrow{\Delta E} [Cu(OH)_2(A_i-A_j)] + 2H_2O$$

were calculated, using the same method as in part 2, and the results are reported in Table 2.

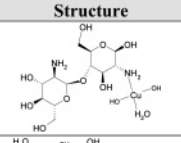
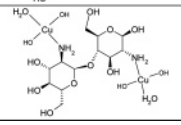
We noticed that the energy of the reaction, ΔE, was located between 51.9 and 151.5 $kJ \cdot mol^{-1}$, and then, these types of complexes were not possible. The bond between metal ions and each monomer induced a highly stressed conformation for the dimer and also involved the loss of several hydrogen bonds and a close angle for the glycosidic bond. These results confirmed the first part of this study: even for the most favorable complex $[(A_{2'}-A_{3'})]$, the conformation of the metal ion and bonded atoms was not so different from that of the model $[(A_{2'3'})]$, and the energy for these two models was not favorable.

Table 2. Energies (ΔE) Calculated for the Different Sites of Coordination of One Cu^{2+} with a Dimer A−A Acting as a Bi-dentate Ligand Noted $[(A_i-A_j)]$[a]

Structure	Complex name	Energy (ΔE) ($kJ.mol^{-1}$)
	$[(A_{2'}-A_{6'})]$	+ 83.2
	$[(A_{2'}-A_{3'})]$	+ 51.9
	$[(A_{6'}-A_{6'})]$	+ 151.5
	$[(A_{2'}-A_{2'})]$	+ 92.0
	$[(A_{2'}-A_{5'})]$	+ 73.5

[a] Each index corresponds to the atom numbering of Figure 1.

Table 3. Energies (ΔE) Calculated when One Ligand A−A Is Coordinated with One Cu^{2+} on the Site 2′ of One A and when One Ligand A−A Is Coordinated with Two Cu^{2+} (One Cu^{2+} for One A on Each 2′ Site) Noted $[Cu(A_{2'}-A_{2'})Cu]$[a]

Structure	Complex name	Energy (ΔE) ($kJ.mol^{-1}$)
	$[(A_{2'}-A)]$	-76.2
	$[Cu(A_{2'}-A_{2'})Cu]$	- 89.7 (by Cu^{2+})

[a] The atom numbering is shown on Figure 1.

We also considered one dimer A−A acting as a bridging ligand with two Cu^{2+} in Table 3. Each copper II ion was binding with the N atom of one amine function (atom 2′ on Figure 1), the most favorable binding site, as seen in part 2. To ensure the neutral squared plan configuration, each coordinating Cu^{2+} was completed by two OH^- and one H_2O. The obtained complex $[(H_2O)(OH)_2Cu-(A_{2'}-A_{2'})-Cu(OH)_2(H_2O)]$ was shortly noted $[Cu(A_{2'}-A_{2'})Cu]$. We proceeded to an energetic optimization with the same DFT method as previously described and the energy was evaluated.

Then, the calculation of the global energy ΔE of the reaction

$$2[Cu(OH)_2(H_2O)_2] + A-A \xrightarrow{\Delta E} [(H_2O)(OH)_2Cu-(A_{2'}-A_{2'})-Cu(OH)_2(H_2O)] + 2H_2$$

Table 4. Orbital Coefficients of Right A (Bold Character) 2′ Nitrogen of a A-A Dimer and a [$A_{2'}$−A] Complex with One Cu^{2+} Ion Bound on the Left A 2′ Nitrogen

atomic orbitals	A−**A**	[$A_{2'}$−**A**]
2s	0.0062	−0.1176
2px	−0.0905	−0.1676
2py	−0.0556	0.1874
2pz	−0.0374	0.4344

was about −179.1 kJ·mol^{-1} for two copper II ions. The interaction energy for one Cu^{2+} was −89.6 kJ·mol^{-1} (Table 3).

It is interesting to compare this result with the global energy ΔE of the reaction

$$[Cu(OH)_2(H_2O)_2] + A-A \xrightarrow{\Delta E} [(H_2O)(OH)_2Cu-(A_{2'}-A)] + H_2$$

In this last complex, noted [($A_{2'}$−A)], a A−A dimer acted as a single pending ligand, and we found $\Delta E = -76.2$ kJ·mol^{-1} as shown in Table 3.

These last two values were found very close to the interaction energy value of one Cu^{2+}, with one A in the monomer pending complex [(A_2′)] (−75.6 kJ·mol^{-1}). However, the difference observed between the interaction energies ΔE, calculated by each Cu^{2+}, for [($A_{2'}$−A)] and [Cu($A_{2'}$−$A_{2'}$)Cu] complexes, is in favor of an increased stability by a second copper ion binding on the second 2′ site of the dimer. These observations seem to suggest that the copper II ion fixation on a chitosan chain should be a cooperative mechanism.

We further examined the highest occupied molecular orbitals (HOMO) of [(A−A)] and [($A_{2'}$−A)] species. The comparison of the orbital coefficients of the amino nitrogens of the HOMOs for [(A−A)] shown in Table 4 revealed that the two nitrogens in A−A were not equivalent: the left 2′ site having higher orbital coefficients than the right 2′ site.

The orbital coefficients of the amine nitrogens of the HOMO for [($A_{2'}$−A)] revealed that the orbital coefficients of the free 2′ nitrogen (right A unit) were enhanced. Therefore, the first copper II ion binding on the left amine site activated a future second binding on the right A amine function. This last observation should agree with a cooperative binding of Cu^{2+} ions on deacetylated sequences of chitosan. This hypothesis will be developed in a deeper manner in the next paper.

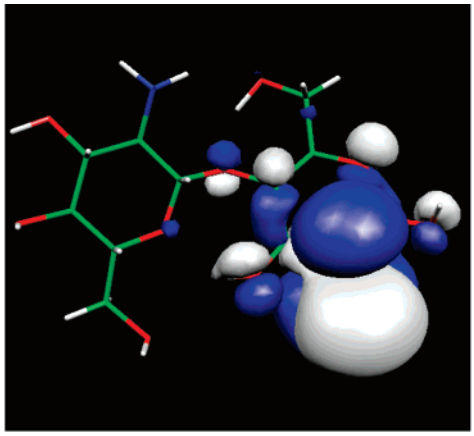
The HOMO orbitals of these two complexes drawn on Figure 4 illustrate these observations.

Moreover, an analysis of the optimized geometry of [Cu($A_{2'}$-$A_{2'}$)Cu] revealed that there were neither interactions nor close contacts between the two Cu^{2+} ions. This suggests that if the chitosan chain is saturated with copper II ions, every amino function should be interacting with only one ion

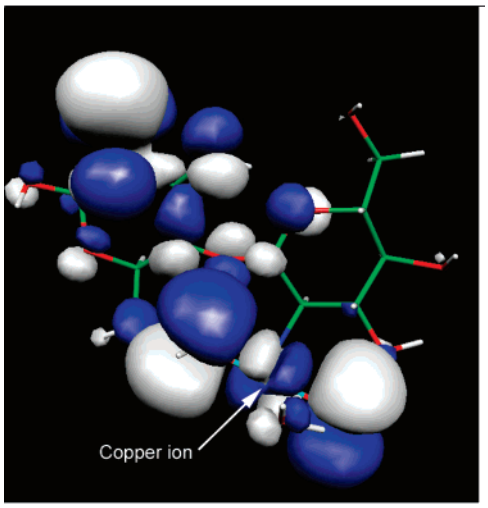
Conclusion

This first theoretical study confirms that, in a weakly alkaline medium, the free amine function of D-glucosamine is the most favorable site for the coordination with one copper II ion leading to a stable pending complex. The heterocyclic oxygen atom of this monomer is another possible site for the coordination with one copper II ion, but the pending complex thus formed is less favorable. All of the other heteroatoms cannot be involved in a coordination with one copper II ion.

One copper II ion can coordinate two glucosamine monodentate ligands with the amino-nitrogen as the coordination site



Homo orbital of the A-A model



Homo orbital of the [A-A_2] model

Figure 4. HOMO orbital of the complex surface computed at a 0.01 density of presence.

and two hydroxyl ion ligands. The so-formed tetracoordinated complex, in the cis conformation, is a little more stable than the previous one, although the trans conformation is more disfavored. On the other hand, the glucosamine residue cannot coordinate as a bidentate ligand with this metal ion.

All of the results of this computational modeling confirm some published results[21,22,23] but definitely desagree with those who proposed the formation of pluridentate complexes between two dimers belonging to two different chains and one copper ion.[24] The possibility to form a cross-linking between two chains throughout a complex involving two different glucosamine residues on each chain and a copper ion remains possible, but this kind of complex is essentially favored in the cis conformation which seems less probable, for steric restrictions on polymer chains. Moreover, the entropic term also plays an important role in the latter case.

No other sites of the *N*-acetyl-D-glucosamine can coordinate with Cu^{2+} ions. The conformational analysis of the four different disaccharides we can build from the two monomers, glucosamine and *N*-acetylglucosamine, confirms the stiffness of chitin and chitosan chains. In accordance with stiffness measurements,[25] glucosamine chains are less rigid than *N*-acetylglucosamine chains. Hydrogen bonding, between amide groups in *N*-acetyl-D-glucosamine and hydrogen atoms of a hydroxyl group belonging to the adjacent monomer, restricts the accessible conformational space and contributes to this stiffness. This

conformational study will be used for the theoretical study of the complexes with longer oligomers of glucosamine and *N*-acetyl glucosamine.

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