

DYNAMIC STUDY OF THE INTERACTION BETWEEN IONS AND A 30 MONOMERS CHITOSAN CHAIN.

*R. Terreux^{*1}, M. Domard¹, A. Domard².*

1 : Laboratoire de Chimie Physique et de Modélisation Moléculaire, Faculté de Pharmacie, Université Claude Bernard, Lyon 1, 8 Avenue Rockefeller, 69373 Lyon Cedex 08, France ;

2 : Laboratoire des Matériaux Polymères et des Biomatériaux, UMR CNRS 5627, Bât. ISTIL, Université Claude Bernard, Lyon 1, Domaine Scientifique de la Doua, 15, Bd. André Latarjet, 69622 Villeurbanne Cedex, France

Abstract (4. Section Heading Style)

Molecular modeling tools are very useful to understand interactions between molecules and /or ions [1]. In this study we are focusing on the interactions between one chain of fully deacetylated chitosan constituted of 30 monomers and counter-ions like ethanoate (CH_3COO^-) or chloride (Cl^-) (each monomer of such chitosan has an amine charged function NH_3^+ bonded to the carbon number 1).

The chain of chitosan was modeled and minimized in energy using the molecular mechanics and the Glycam Forcefield. This chain was inserted in a parallelepiped box and filled with solvent and 30 counter-ions. A dynamics simulation was performed with AMBER 8 (molecular modeling package using the Glycam forcefield) at constant temperature 300 K and constant pressure (1 atm) for 10 ns.

This procedure was applied for two solvents successively: first, pure water (TIP5P water model molecule) then a mix of 50 % water (TIP5P water model molecule) with 50 % propan-1,3 diol (percentage in mass).

Two kinds of counter-ions were studied successively in each solvent: first 30 chlorides Cl^- , then 30 ethanoates (CH_3COO^-).

In a complement of these four simulations, we studied the competition between chloride and ethanoate ions: we put 15 chlorides and 15 ethanoates in each previous solvent.

Therefore, six dynamic simulations were performed, each one leading to obtain the trajectories of all the atoms of the system during 10ns. These 6 simulations were deeply analyzed. The number of contact and the distribution of each ion around the chain reveal different behaviors for the chloride and the ethanoate ions. These theoretical calculations confirm experimental experiences, and increase the knowledge of the interactions between ions and chitosan chains.

Introduction

Interactions between chitin, chitosan, or their derivatives and ions are of great interest and are particularly important in the fields of bioorganic chemistry¹. D- Glucosamine and small co-oligomers of D-glucosamine and *N* acetyl-D-glucosamine can also form stable complexes with their counter-ions. The complexing behavior of these amino-sugars (monomers, oligomers or polymers) is still not fully understood². Although it is generally accepted that free amino groups could be involved in the coordination with heavy metal ions, possible complexes between glucosamine in the ammonium form and its counter-ions, especially the participation or not of hydroxyl groups in the ion coordination has not been really investigated yet.

To clearly answer the question about the active sites of complexation and the detailed structure of complexes formed with counter-ions, we planned a theoretical approach of the problem. We used molecular mechanics methods to describe the whole system, a 30 charged D-glucosamine monomers chain with 30 anionic counter-ions, in various solvents. The global charge of the system was neutral. Two different ions were studied: chloride and ethanoate ions. Two solvents were also investigated: water and a 50/50 in mass mixture of water and 1,2-propandiol. With each solvent three cases were considered: 30 chlorides and 1 chain, then 30 ethanoates and 1 chain and finally, 15 chlorides and 15 ethanoates with 1 chain. A total of 6 molecular dynamics simulations were computed.

Construction of models

The 30 charged monomers D-glucosamine chain was constructed in its minimal energy state. The θ and ϕ dihedral angles were chosen³ to have a minimum torsion energy between two monomers. The chair conformation of each monomer was controlled after the construction of the chain. As a consequence the chain was in a linear conformation with charged amino groups distributed alternately on both sides of the chain. 30 ethanoate ions were randomly placed around the polymer with a minimum distance of 13 Å. For the other simulations with the chloride ion, in order to have the same reference point, we started from the ethanoate system and mutated these ions into chloride. For the last two simulations with 15 chloride and 15 ethanoate ions; 15 ethanoate ions were randomly chosen and mutated into chloride.

For each study this 30 monomers chitosan chain with its 30 counter-ions was placed in an equilibrated parallelepiped box of solvent molecules. A periodic boundary condition (PBC) was applied: when one solvent molecule went out from the box by one face it was reintroduced by the opposite face.

Two different solvents were studied, water and the mixture water/1,2-propandiol (50/50 w/w). The water molecule model selected was the TIP5P⁴. This model with two dummy atoms simulates the role of the lone pairs and leads to have an excellent description of the hydrogen bonds. For the simulation in water solvent we chose the pre-equilibrated box of TIP5P water molecule included in the AMBER 8 molecular modelling package⁵. For simulation in solvent mixture, a box was created with one 1,2-propandiol surrounded by 4 TIP5P water molecules. The mass of the 1,2-propandiol is equivalent of the 4 water molecules to have a 50/50 ratio in mass. Partial charges of the atoms of the 1,2-propandiol were computed by quantum chemistry using Density Functional Theory methods (DFT). The B3LYP methods with the atom centred charged algorithm and the 6-31G**molecular basis set were used on the Gaussian 98A10 program⁶. The computed partial charges of the 1,2-propandiol are adjusted to be equivalent to partial charges of TIP5P water molecule in order to prevent heterogeneous mixture. The box was energetically minimized and the resulting structure duplicated in a three dimensions space to create a large cube of about 30 Å edge long. A 2 ns molecular dynamics simulation using the AMBER 8 program was performed at constant pressure (1 bar) and temperature (300K). The trajectories of the atoms were analysed in order to verify the stability of the homogeneity of the mixture.

For each study the solvent box dimension was increased of 15 Å in the three dimensions of space to avoid interactions between the chitosan chain in the box and its replicated images introduced for the PBC. For each model a 10 ns molecular dynamics calculation at constant pressure (1bar) and constant temperature (300K) using the AMBER 8 program were computed on the 32 cpu parallel supercomputer of the LCPM² laboratory. The GLYCAM force-field⁷ was used for this simulation. This force-field was particularly well adapted for carbohydrate simulations.

Results and discussions

The analysis of the trajectories of atoms of the chitosan chain and ions during 10 ns was performed and several parameters were measured. The same analysis was processed for the six simulations. The analysis of temperature and the different energy terms revealed that simulations were stable and each of them reached the equilibrium in less than 0,5 ns. The distances between each ions and each charged amino function were measured during the 10 ns. The distance for each ion to the closest amino site was measured during all the simulation. The analysis was performed for all ions during the simulation time. These data were particularly difficult to analyse due to the great variation of distances for each ion. We defined five classes as a function of the distances between ions and the chitosan chain. The percentage of ions in each class was counted during the simulation. The partitions of the distance of the counter-ions to the chain for all simulations are reported on figure 1. If the distance between one ion and the chain is less than 5 Å, this means that the ion is directly in contact with the chain. Using this partition, the mean number of contacts and the mean duration of contacts were computed for each ion.

The analysis reveals a difference of behaviour between chloride and ethanoate counter-ions. On table 1 are reported the mean times of contact.

Chloride and ethanoate ions during the observed simulation duration (10 ns) had a similar number of contacts with the chitosan chain, although slightly lower for ethanoate. The different solvents did not really affect this number of contacts. If we consider now the duration of contacts, we notice a small variation with the solvent with times a little lower in the alcohol. On the contrary, a great difference between the duration of contacts between chloride and ethanoate in the same solvent was evidenced with about 30 ps for chloride compared to 250 ps for ethanoate ions.

These data seem to suggest that the binding energy is higher for ethanoate ions, than chloride. This can be only explained by the formation of hydrogen bonding. We can see on the trajectories of atoms that the carboxylic acid functions of ethanoate ions are in contact with the charged nitrogen sites of the chitosan chain, which comforts the involvement of the hydrogen bonding. Since dynamics simulations are based on the molecular mechanics theory, they cannot allow a fine description of hydrogen bonds. Indeed, in this theory, hydrogen bonds are mostly described as electrostatic interactions⁸.

In order to confirm this assumption, quantum calculations were performed. A model of chloride ion in interaction with the charged amino function of one monomer of D- glucosamine was minimized using the Density Functional theory method B3LYP and the atomic basis set 6-31G**. A model with ethanoate ions in a monodentate interaction with the charged amino function was processed and minimized using the same methods. This complex was unstable and, we rapidly observed the ethanoate ion establishing a second hydrogen bond with the hydroxyl function of the carbon number 3 (according to the classical numbering⁹). Quantum calculations were made in a gas phase and the formation energy was -587 kJ.mol⁻¹ for chloride and -643 kJ.mol⁻¹ for the ethanoate. The difference of binding energy was 66 kJ.mol⁻¹ and thus, two hydrogen bonding interactions were involved for the ethanoate counter-ion. Then, the difference of about 33 kJ.mol⁻¹ by bond agreed with the range of hydrogen bonds energy. These calculations demonstrate clearly that ethanoate ions make 2

hydrogen bonds with a glucosamine residue in a chitosan chain. A similar calculation was made on a dimer of chitosan in interaction with one ethanoate ion. The ion was initially set to make a hydrogen bond with the amino function of the first monomer and a second hydrogen bond with the hydroxyl function 6' of the second monomer. The model was unstable and we could observe that after a few iterations the ethanoate migrated to one monomer as in the monomer model. Therefore ethanoate ion could not make a bridge between two consecutive monomer units in a polymer chain.

According to the above analysis the ethanoate ion has a higher affinity for the chitosan chain than chloride. Effectively we observed that the percentage of repartition of binding ions (figure 1) was slightly lower for chloride ions (5%) than for ethanoate ions (10%) in aqueous solution. The same percentages in the solvent mixture were about 8% for chloride and 30% for ethanoate showing first that the behaviour was independent of the solvent and secondly that hydrogen bonding was certainly reinforced by the presence of a solvent of lower dielectric constant than water.

An analysis of the chitosan chain during simulation revealed that the chain conformation was like a fluctuant broken line from which we could extract the persistence length. From the trajectories of the atoms of the chain, we could calculate the root mean square of the displacement (RMSD) of heavy atoms of selected monomers (i-j), where i and j were the numbers of the monomers on the chain, varying from 1 to 30.

We proceeded to the analysis in two steps. In the first one, the conformations of the chain was aligned on the three central monomers (14-16) and the RMSD were computed on 15-X, X varying from 1 to 30. The results are plotted on figure 2 (upper left). The variation of the RMSD (Δ RMSD) was also calculated and plotted on figure 2 (down left). The plot of the RMSD measured from the central monomer to the considered one, revealed two breaks: one close to the monomer 11 and the second on the monomer 21. It suggested that the persistence length corresponded to about the contour length of 10 consecutive monomers. In order to validate this result, we proceeded to a second step: we aligned the chain on monomers between 10 and 20 and did the same RMSD and Δ RMSD calculations. The results were plotted on figure 2 right side. We clearly observed the first break on the monomer 11 and the second on monomer number 21. This second step confirmed the first evaluation of the persistence length: 10 successive monomers or a distance of 55 Å. The same analysis was performed on the three other simulations; the nature of ions and solvent did not change the persistence length. The constancy of the persistent length for ethanoate or chloride confirmed the binding scheme of the ethanoate ions on one monomer only.

The persistence length obtained from this method of computation agrees quite well with values obtained experimentally¹⁰.

Conclusion

Our results obtained by molecular dynamics and quantum mechanics are particularly interesting to interpret some experimental results. They first demonstrate that due to the difference of mechanism of interaction between chloride and ethanoate ions, solution properties of chitosan necessarily depend on the nature of the counter-ion. They also show that the persistence length of chitosan but also of other polymers could be determined by molecular dynamics.

These two techniques have to be tested on other counter-ions and other polymers.

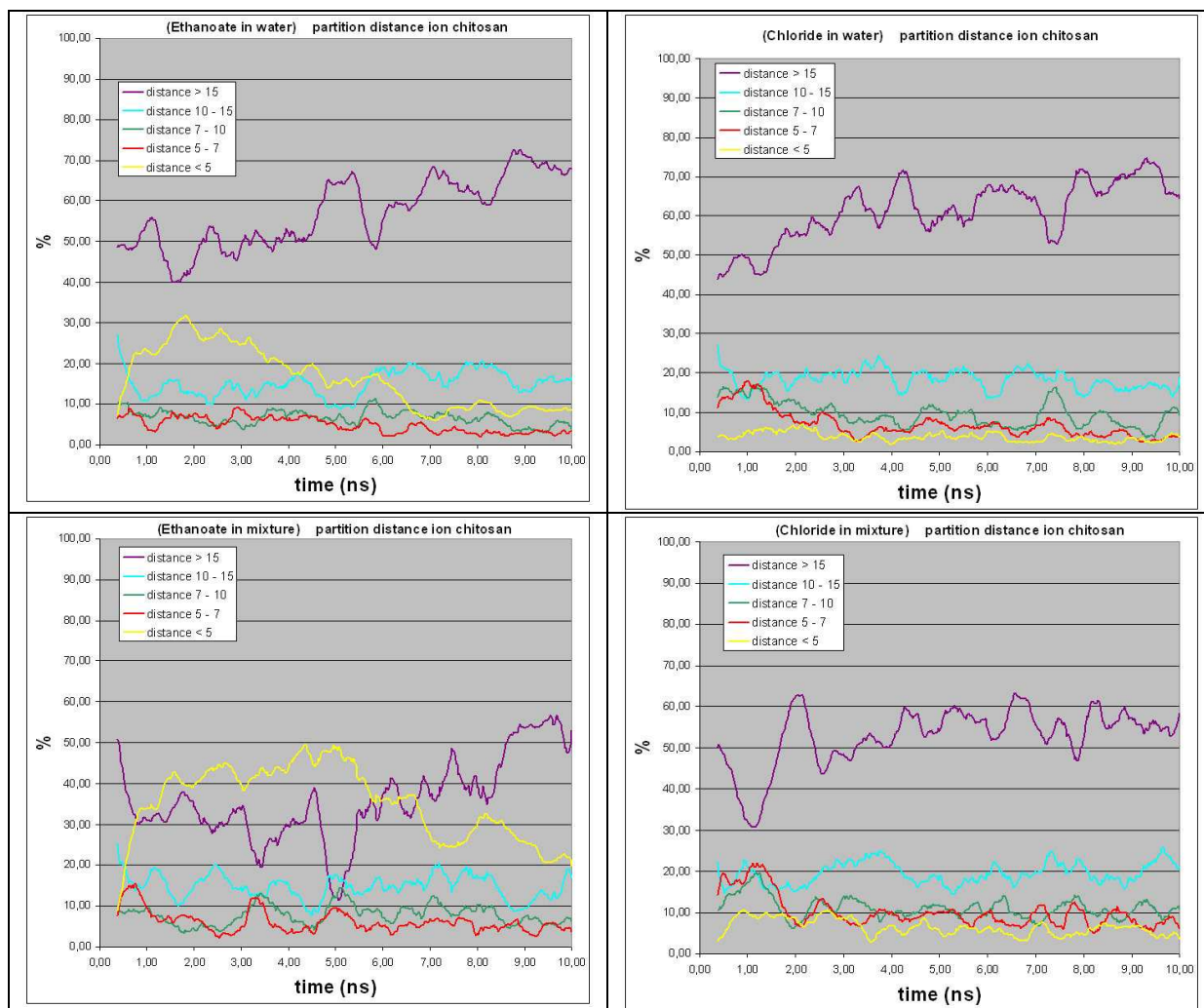


Figure 1 : partition of the distance ion to the chain.

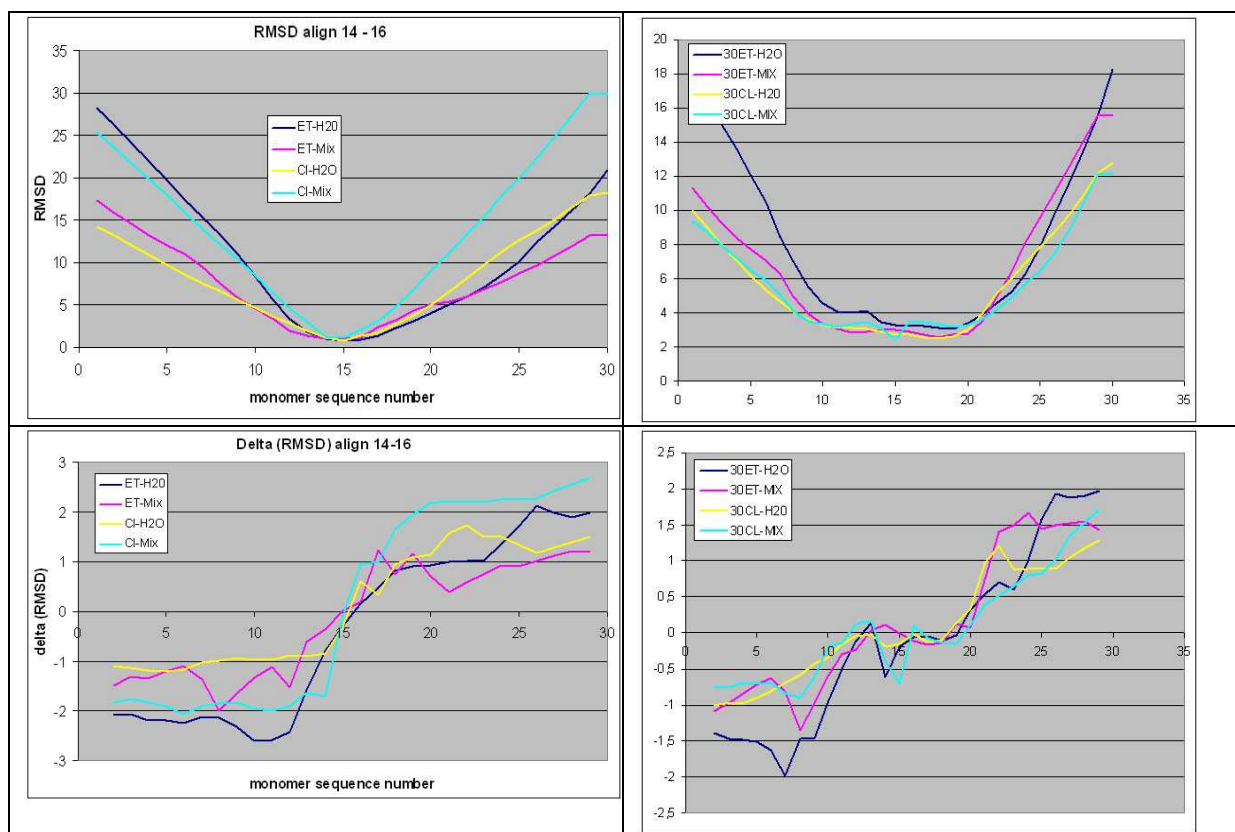


Figure 2 : RMSD and Delta RMSD on the chain of chitosan.

| | Mean duration of contacts (ps) | | Mean number of contacts during 10 ns | |
|--|--------------------------------|-----------|--------------------------------------|-----------|
| | Chloride | Ethanoate | Chloride | Ethanoate |
| 30 same ions in water | 25,6 | 151,5 | 14,5 | 10,5 |
| 30 same ions in the solvent mixture | 35,8 | 231,2 | 17,6 | 14,9 |
| 15 chloride and 15 ethanoate ions in water | 29,0 | 220,7 | 16,1 | 13 |
| 15 chloride and 15 ethanoate ions in mixture | 31,6 | 408,4 | 11,5 | 8,1 |

Figure 3 : mean time of contact

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