SHORT COMMUNICATION

Investigation of Chitosan, Quercetin and Water Interactions using Free Energy Calculations and Molecular Docking

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Abstract:

Predicting the preferred orientation of a ligand to a receptor and the free energy (ΔG_{AB}) of these molecules in a solution is very useful for pharmaceutical applications. In this work, ΔG_{AB} between chitosan and quercetin molecules with water were obtained by the thermodynamic integration (TI) method and the chitosan+quercetin complex was investigated by Molecular Docking (MDK). The topology of the molecules was compatible with the CHARMM force-field. Both molecules were solvated with the GROMACS SPC water model. The ΔG_{AB} values of the molecule+water systems with the thermodynamic integration (TI) method was done in a 10 ns simulation. The complex was achieved in a 2.5 nm x 2.5 nm x 2.5 nm box. The ΔG_{AB} values for the molecules+water systems were 14.36 +/- 0.46 and 0.19 +/- 0.53 kJ/mol, respectively. For the complex, nine conformations were obtained for the quercetin around the chitosan. The best conformation showed an affinity energy of -3.8 kcal/mol. The ΔG_{AB} results for the systems confirm the low or no solubility of the molecules in the water under normal conditions, as already demonstrated experimentally. The docking showed that the interaction between chitosan and quercetin molecules involves hydrogen bonds in a specific position.

Keywords: chitosan; free energy calculations; molecular docking; quercetin

1. Introduction

Some therapeutics agents are unstable and need protection against degradation due to a biologic environment. Thus, the development of new and improved materials for protecting these agents from degradation is of great importance, especially those from biopolymers [1–3]. These systems are formed by a polymeric array that carries the therapeutic agent, adapting the pharmacological velocity of liberation [4].

A candidate for polymeric support for drug delivery is chitosan, being a biodegradable and biocompatible polysaccharide that has low toxicity and mucoadhesive properties. Chitosan can also be degraded under the action of microorganisms, thus being easily removable from the body [4, 5]. Chitosan is a biodegradable polymer composed predominantly of repeat units of glucosamine or acetylglucosamine, depending on the degree of deacetylation, which can range from 50% to 98% [4, 5].

Quercetin, a potential therapeutic agent, is a flavonol ubiquitous in plants and one of the main micronutrients present in food, being available through the ingestion of fruits, vegetables, and some types of drinks [6, 7]. An antioxidant compound, quercetin has health-promoting effects, such as the improvement of cardiovascular health, as well as the reduction of some cancer risks and inflammatory disorders, as shown by some epidemiological studies [6, 7]. However, because of a low solubility in water and being an unstable compound in a physiological environment, it requires an alternative means for an improvement of its bioavailability [6, 7]. In this context, a controlled release is a means of overcoming this

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disadvantage.

To investigate controlled-release systems and their potential as drug-delivering systems, free-energy calculation (ΔG_{AB}) and molecular docking (MDK) are useful tools. The free-energy calculation can determine how a process will proceed together with the probability of the system adopting a given state, via which it may be possible to obtain information such as the binding of ligands to macromolecules, solubility, the partitioning of drugs, etc. [8]. This information can be allied with MDK studies, because in this type of simulation, the different spatial conformations of the ligand are obtained, which allows for the identification of the most probable conformation in the binder and protein (or molecule) interaction [9]. In this work, free-energy calculations were conducted to study the interactions between chitosan and quercetin with water; the chitosan+quercetin was obtained by MDK.

2. Results and Discussion

In the RHF method, the charges of the Löwdin population [10] replaced the original charges present in the generated ITP files, made possible by using the MINI base group [11, 12]. The free-energy calculations generated ΔG_{AB} of 14.36 ±0.46 and of 0.19 ±0.53 kJ/mol for chitosan and quercetin, respectively. These results seem to be consistent with experimental results on the solubility of both chitosan and quercetin. Is well known that chitosan its only soluble in pH between 6.0 e 6.5 [4]. Works like Kumar et al [5], showed a lot of studies that confirm the insolubility of chitosan in water at normal conditions. Chebil et al. [13], reported 0.01 g/L as the aqueous solubility of quercetin at 20ºC. Otherwise, a number of the free energy of these molecules in water was not experimentally reported until this work was done.

To understand the behavior of chitosan and quercetin in the hydrophilic solution, the $atom_{molecule}$ and $atom_{water}$ interactions were analyzed.

Figure 1a shows three crucial interactions. Firstly, the interactions of chitosan between the oxygen atoms (in red) of OH groups with the hydrogen atoms (in light gray) of water; secondly, the interactions of the oxygen atoms of the ring of the chitosan molecule with the hydrogen atoms (in light gray) of the water molecule; and, thirdly, the interactions of the chitosan hydrogen atoms (in light gray) of their amine groups with water's oxygen atoms (in red). Interactions between oxygen and hydrogen atoms indicate a hydrogen-bonding class. This occurs due to the fact that the oxygen and nitrogen atoms are more electronegative than carbon, so the carbon bound to one of these atoms will have a positive partial charge (δ^+).
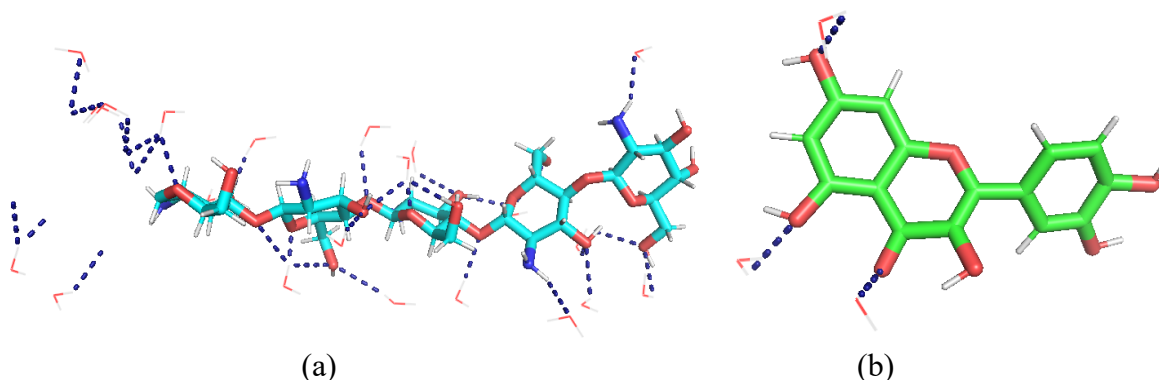

(a)   (b)

Figure 1. Interaction between (a) chitosan atoms and water atoms and (b) quercetin atoms and water atoms.

Figure 1b shows quercetin's interactions between the oxygen atoms (in red) of OH groups with the hydrogen atoms (in light gray) of the water molecule. It is possible to observe that the solvent atoms tend to gather around the benzene rings, due to the non-bonding electron pair (with partial positive charge δ^+) of the oxygen atom present in the OH group. The presence of the

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hydroxyl group enhances the compound's ability to form strong intermolecular hydrogen bonds. This effect gives a modest solubility in water to molecules with phenol groups. This quercetin molecule behavior is due to the carbons of the phenyl ring, as these work to form its hydrophobic part while the hydroxyl groups form their polar portion [15]. It is understood that there are hydrogen bonds between the quercetin and the water.

This study shows that these interactions between water (solvent) and molecules (solute) occur through hydrogen bonds; therefore, how soluble these molecules are in water must be related to the intensity of these hydrogen interactions and also to their radial distances, which will be analyzed posteriorly. The study also shows that the interactions occurring between molecules and water are interactions related to the partial charges of their atoms, as well as that the atoms with such charges were the atoms O and H characterizing the hydrogen bonds.

The docking of the chitosan+quercetin system generated nine different conformations for quercetin (chosen as a ligand) around the chitosan molecule. Table 1 shows different values for the binding affinity and for the deviation of the root mean square (RMSD). To generate the most stable complex for future interaction studies with water and 1-octanol, the choice should take into account the binding affinity and RMSD values. The RMSD value of 0.000 for conformation 1 indicates its use as a reference for the calculation of deviation in quercetin conformations in relation to chitosan. The conformations 5 and 8 have a low deviation from the conformation 1, while the conformations 2, 3 and 7 greatly change their conformations around the chitosan when compared with the conformation 1.

From Table 1, it can be seen that the conformations 1 and 2 presented the most negative binding affinities, both with -3.8 kcal/mol and both involving oxygen and hydrogens in their interactions – with the hydrogen interactions shown in Figure 2.
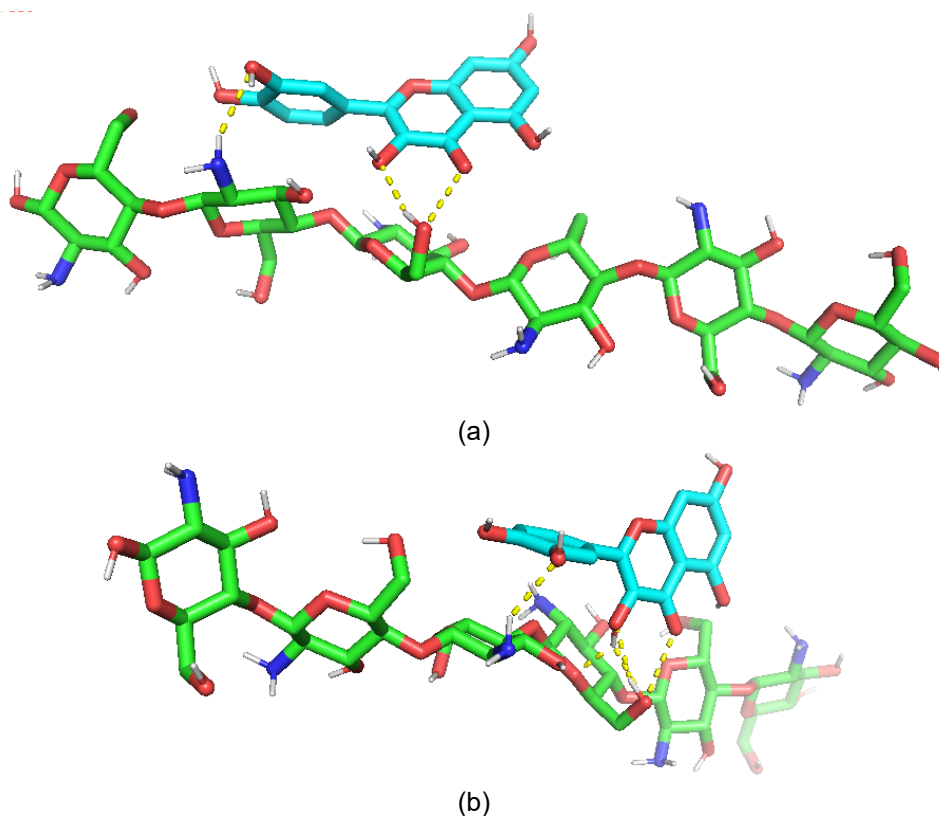

(a)

(b)

Figure 2. Conformations of quercetin around chitosan.

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Table 1. Bound affinity of the conformations in the DM.

Conformation	Bound affinity (kcal/mol)	RMSD
1	- 3,8	0,000
2	- 3,8	9,358
3	- 3,7	9,704
4	- 3,7	8,642
5	- 3,7	1,923
6	- 3,7	8,533
7	- 3,6	9,630
8	- 3,6	1,928
9	- 3,6	3,753

However, by the RMSD values, the conformation 2 is considerably different from the conformation 1. For the quercetin around the chitosan, the conformation 1 has the O-6 (oxygen) atom of quercetin interacting with the atom 1HN2 (hydrogen) of the chitosan (interaction 1), the atom O-5 (oxygen) of the quercetin interacting with the HO-6 (hydrogen) atom of chitosan (interaction 2), and the O-1 (oxygen) atom of quercetin interacting with the HO-6 atom of chitosan (interaction 3). Interactions 1 and 2 indicate hydrogen bonds between molecules, with interaction 2 being a weaker dipole-induced physical interaction because it involves an oxygen bound to an OH group and oxygen attached to a ring. The conformation 2 has interactions involving: the quercetin O-6 (oxygen) atom and the 1HN2 (hydrogen) atom of chitosan (interaction 4), the H-8 (hydrogen) atom of quercetin with the O-5 (oxygen) atom of chitosan (interaction 5), the O-5 (oxygen) atom of quercetin and the HO-6 (hydrogen) atom of chitosan (interaction 6), and the O-1 (oxygen) atom of quercetin with the HO-6 (hydrogen) atom of chitosan (interaction 7). All interactions of conformation 2 are hydrogen bonds.

We can consider that both conformations of the quercetin molecule can occur naturally in a chitosan+quercetin complex because their binding affinities are the most negative. In this sense, a complex was done with the conformation (a) and another with the conformation (b) to aid subsequent studies of the complex in hydrophilic and lipophilic environments.

3. Material and Methods

The three-dimensional structure of chitosan was designed using the web-based molecular builder SWEET [16], while the three-dimensional structure of quercetin (ZINC03869685) was obtained by the ZINC database.

Both structures were designed to obtain their optimized partial charges. The calculations were made using the GAMESS/US 2014-12-05 [17] software for Linux 64 bits, and by the Restricted Hartree-Fock (RHF) method using the MINI base group in 500 steps [11, 12]. The topology of the molecules was generated by the WEB server SwissParam [18], which is compatible with the CHARMM force-field [11, 12]. The WEB server also produced the GRO files, which were compatible with GROMACS 5.0.2 [19] software for Windows 64 bits, which were used to produce GRO files of solvated molecules. The solvation of the molecule+water systems was carried out with the GROMACS SPC water model in a dodecahedral box with a minimum distance of 1.00 nm.

In order to calculate ΔG_{AB} of the molecule+water systems we used the integration method with 21 points λ: 0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55, 0.60, 0.65, 0.70, 0.75, 0.80, 0.85, 0.90, 0.95, 1.00, calculated by the GROMACS software. For this, each point λ underwent a minimization of energy by the steepest descent minimization method, together with the L-BFGS method and equilibration at a constant temperature of 300 K and with a constant pressure of 1 bar [20, 21]. The files for analysis were obtained by the gmx bar module also using the GROMACS software [22, 23].

The chitosan+quercetin complex was obtained by DMK using the virtual-screening software PyRx 0.8 [24], for Windows 64 bits, compatible with the AutoDock Vina responsible for the docking calculations made in a 2,5 nm x 2,5 nm x 2,5 nm box size.

4. Conclusions

The free-energy calculations for the chitosan and quercetin molecules in a hydrophilic environment were consistent with experimental results on the solubility of the molecules in water. These energies in water have positive values showing that chitosan and quercetin should not dissolve in water under normal external

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conditions. However, quercetin has a higher affinity for water than chitosan, which may indicate that when a complex of these two molecules is in a hydrophilic environment the quercetin will have a preference by water and will detach itself of the chitosan, allowing it to then have a release of the quercetin. It is possible to produce the chitosan+quercetin complex by means of DMK, enabling an analysis of the interaction of this complex in water as well as of its possible uses as a drug-delivery system. Because the interactions between the two molecules occur through hydrogen bonds, it is possible that chitosan can be used as a polymeric support for quercetin. This is because the hydrogen bonds are weak enough to not alter the chemical properties of the molecules, transforming them in a new compound, and strong enough to hold the complex together.

Acknowledgments

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