

Semiempirical ΔH_{bind} calculations for interactions between the RTA and RTB subunits and ricin inhibitors

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Abstract: Industry, governments and the media have become increasingly more interested in the castor bean seeds (Ricinus communis L.). This stems from the unusual properties of its byproducts, such as castor oil and ricin. The ricinoleic acid comprises around 90% of all fatty acids extracted from the castor bean plant [1].

Besides the interest in its oil, the co-products generated during its production have garnered wide commercial attention. This is due to the production of around 1.5 million tons per year [1]. Hence, it is in the best interest of the industry to find an economically viable purpose for these co-products. A welcoming alternative regards the use of these co-products as animal food, though it is still not possible due to the presence of ricin, a ribosome-inactivating protein composed by two sub-units (known as RTA and RTB), in which the RTA serves as the catalytic sub-unit [2]. In addition to the problems related to co-products in the production of castor oil, terrorist groups yet utilize the ricin as a chemical weapon [3]. In this manner, the inhibition of the action mechanism within the ricin is of major economic, public and military interest, with the RTA being the target of inhibitors. Recently, fields of study within theoretical and computational chemistry have developed a capital role in the research of biological and/or biochemical systems, which provide with a proper orientation towards the conception of new drugs.

This study has carried out calculations for enthalpy of formation (ΔH_f) and ground state geometries of RTA and RTB subunit, both separately and joined to form complexes containing possible inhibitors, through semiempirical methods such as: RM1, PM6, PM6-DH+ and PM7. Crystallographic structures available at the PDB(ID) of the complexes containing inhibitors (0RB, PT1, EJ5, JP2) and of the RTA and RTB Ricin subunits (2AAI) were used in these studies. We also performed studies with two different inhibitor candidates synthesized by the group (Lv213 and Lv215). The structures were positioned in the active site of RTA through *Molecular Docking* [4]. The objective was to identify mechanisms that would favor ricin inhibition and to verify which semiempirical method would better describe the binding enthalpies (ΔH_{bind}) of the RTA-ligand and RTA-RTB complexes, at least from a qualitative viewpoint.



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Semiempirical calculations of ΔH_f for ligands (0RB, PT1, EJ5, JP2, Lv213 and Lv215), RTA-0RB (4228 atoms), RTA-PT1 (4250 atoms), RTA-EJ5 (4234 atoms), RTA-JP2 (4218 atoms), RTA-Lv213 (4245 atoms) and RTA-Lv215 (4238 atoms) complexes were performed using the MOZYME [5] linear scaling technique implemented on the MOPAC program [6]. We carried out these calculations with the crystallographic structures, optimized by each of the mentioned methods. For the ricin structure (2AAI), since the RTB subunit presents glycosylations in its structure, ΔH_f calculations were conducted for the RTA-RTB systems without glycosylations (8212 atoms) and RTA-RTB with glycosylations (8444 atoms). Once the data for the ΔH_f of the ligands, RTA, RTB and the RTA-ligands and RTA-RTB complexes were calculated, one obtains the $\Delta H_{(bind)}$ values for the RTA-RTB systems (without glycosylations), RTA-RTB systems (with glycosylations) and the various RTA-ligands complexes. In all calculations, we considered the effects of the solvent through the implicit COSMO model for proteins solvated in water.

The ΔH_f results on the crystallographic geometry using the PM7 semiempirical method presented the following values for the RTA-ligand complexes: RTA-0RB2 $[\Delta H_{(bind)}] = -61.04 \text{ kcal/mol}]$, RTA-1PT $[\Delta H_{(bind)}] = -62.04 \text{ kcal/mol}]$, RTA-EJ5 $[\Delta H_{(bind)}] = -69.23 \text{ kcal/mol}]$, RTA-JP2 $[\Delta H_{(bind)}] = -60.39 \text{ kcal/mol}]$, RTA-Lv213 $[\Delta H_{(bind)}] = -32.31 \text{ kcal/mol}]$ and RTA-Lv215 $[\Delta H_{(bind)}] = -17.62 \text{ kcal/mol}]$. All values are consistent with those were experimentally observed for common enzyme-ligand complexes (at least in terms of the value range). Therefore, the data shows that, from an enthalpy viewpoint, the EJ5 ligand presented the lowest $\Delta H_{(bind)}$ when forming a complex with the RTA. On the other hand, the Lv215 ligand was the one presented the highest interaction value. Another interesting point is that the RTA-RTB complex without glycosylations presented an unfavorable interaction enthalpy ($\Delta H_{(bind)} = 80.91 \text{ kj/mol}$) for its formation. However, the RTA-RTB complex with glycosylations presented a very pronounced favorable interaction ($\Delta H_{(bind)} = -6568.311 \text{ kj/mol}$). Such results suggest the glycosylations play an important role in the formation of the ricin enzyme complex (RTA-RTB).

Key-words: Ricin, semi-empirical methods, enzyme-ligand interaction energy

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