MOLECULAR DYNAMICS STUDY ON THE INFLUENCE OF INHIBITORS IN THE DIMERIC INTERFACE OF THE *Tc*DHODH ENZYME

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Introduction

Chagas disease represents a significant public health problem. There is no effective treatment against this parasitic disease, especially in the chronic phase, and its evolution can lead to disability in infected individuals, in addition to about 10,000 deaths per year.[1] In search for new drug candidates with less adverse effects and better therapeutic response in the chronic phase of the disease when compared to current drugs, researchers have been conducting research for selective biological targets for the disease-causing agent, the protozoan *Trypanosoma cruzi*. Among these, the enzyme dihydroorotate dehydrogenase (DHODH) stands out for being essential for the parasite, participating in the synthesis of DNA and RNA, membrane biosynthesis, and other events that occur in cell metabolism.[2]

The computer-aided drug design (CADD) techniques have been consolidated as important tools in this process, allowing cost reduction and increase the chance of achieving a new drug. Molecular dynamics is a CADD technique capable of simulating the behavior of atoms present in molecules or even individually through computational calculations based on molecular mechanics.[3]

The *Tc*DHODH enzyme has an alternating mechanism of action, where the active site of a dimer subunit is opened for substrate entry and exit, concurrently with the closing of the other subunit for the reaction to occur. Reis *et.* al[4] proposed a mutation in *Tc*DHODH class 1A in the Gly138 residue resulting in the loss of interaction with the Asp170 residue of the adjacent subunit and inactivation of the opening/closing movement of the active site. Therefore it becomes interesting further investigations on the mechanism of action of inhibitors on the interface.

Material and Methods

The work was carried out from the following complexes, with PDB code: 2E6F (R = 1.26 Å)[5], ligand OXC (pK_i 4,66 M) and 3W7D (R = 1.52 Å)[6], ligand W7D (pK_i 7,34 M). The molecular dynamic simulations of the protein and its cofactor (flavin mononucleotide) and the ligand complexes were performed in NPT ensemble for 50 ns at 310 K in 1 bar pressure, using program GROMACS 2021. Molecular structures schematics were presented by program Chimera 1.13.1 and all molecular dynamics simulations trajectory analyses were performed using programs in GROMACS 2021 package.

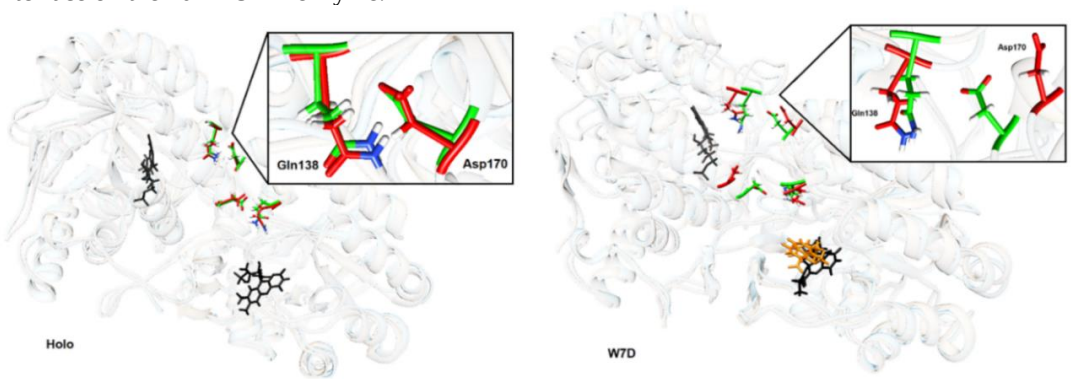
Results and Discussion

The analysis of the simulation was carried out during the 50 ns of simulation, enabling the determination of the equilibration time to the system that reached the stability state after the 4 ns of simulation.

In simulations in the absence of the ligands, it was observed that the Gly138A-Asp170B amino acid residues in the protein carry out 5 hydrogen bonds, which persisted for more than 14% of the time as follows: N-OD1 (85.6%); N-OD2 (76.3%); NE2-OD2 (62.4%); NE2-OD1 (49.4%) and NE2-N (14.6%). However, in the presence of the OXC ligand, only the H-bond interactions NE2-OD1 (18.6%) and NE2-OD2 (17.7%) were maintained, but with less persistence time, indicating the loss of this communication at the interface for the W7D ligand and loss of the H-bond N-OD1 (15.5%).

The water-mediated H-bonds of these residues (Gly138A-Asp170B) were also measured by holo-dynamics, showing a greater number of H-bonds as well. Among them, seven interactions persisted for more than 10% of the simulation time, ranging from 10.89% to 59.41%, five with the Gln138 residue and two with the Asp170 residue. In the dynamic simulation with the OXC ligand, only three H-bond interactions were observed that persisted for 10.37%, 20.80%, and 39.90% of the time, while with W7D only two persisted for 13.10% and 19.68%. These results indicate that the presence of a ligand and how it binds to the active site of the *Tc*DHODH enzyme can disturb the H-bonds on the dimer interface.

Figure 1. Inhibitor-mediated loss of communication between Gly138A and Asp170B residues of the dimer interface on the *Tc*DHODH enzyme.



Conclusion

Analysis of the hydrogen bond interactions between the amino acid residues on the *Tc*DHODH dimer interface demonstrated that the presence of an inhibitor is capable of disturbing these interactions, resulting in a possible loss of subunit communication. Thus, forward studies should be developed to investigate these results as a putative inhibition mechanism of this enzyme.

Acknowledgments

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