DIHYDROOROTATE DEHYDROGENASE INHIBITOR INFLUENCE ON THE ACTIVE LOOP

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Introduction

Chagas disease represents a serious public health problem. There are around 7 million infected people wordwide, causing an average of 14.000 deaths per year, mainly in Latin America where the disease is endemic. Brazil is the country where Chagas disease is more present, with 4.6 million people infected.[1] There is no effective treatment against this parasitic disease, especially in the chronic phase. Therefore, the search for new drug candidate with a better therapeutic response in the chronic phase of the disease and with fewer adverse effects is of great importance. Researchers have conducted studies to find selective biological targets for the disease-causing agent, the parasite *Trypanosoma cruzi*. Among there, the enzyme dihydroorotate dehydrogenase (DHODH) can be an important target for being selective, minimizing adverse effects and for being essential for the maintenance of the parasite's life. The DHODH enzyme participates 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 to achieve 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 DHODH enzyme is formed by a dimer and its mechanism of action occurs with the opening and closing of an active loop in an alternately.[4] Thus, it becomes interesting to observe the behavior of the enzyme against ligands with different inhibitory potentials, if changes occur in the movement of the active loop.

Material and Methods

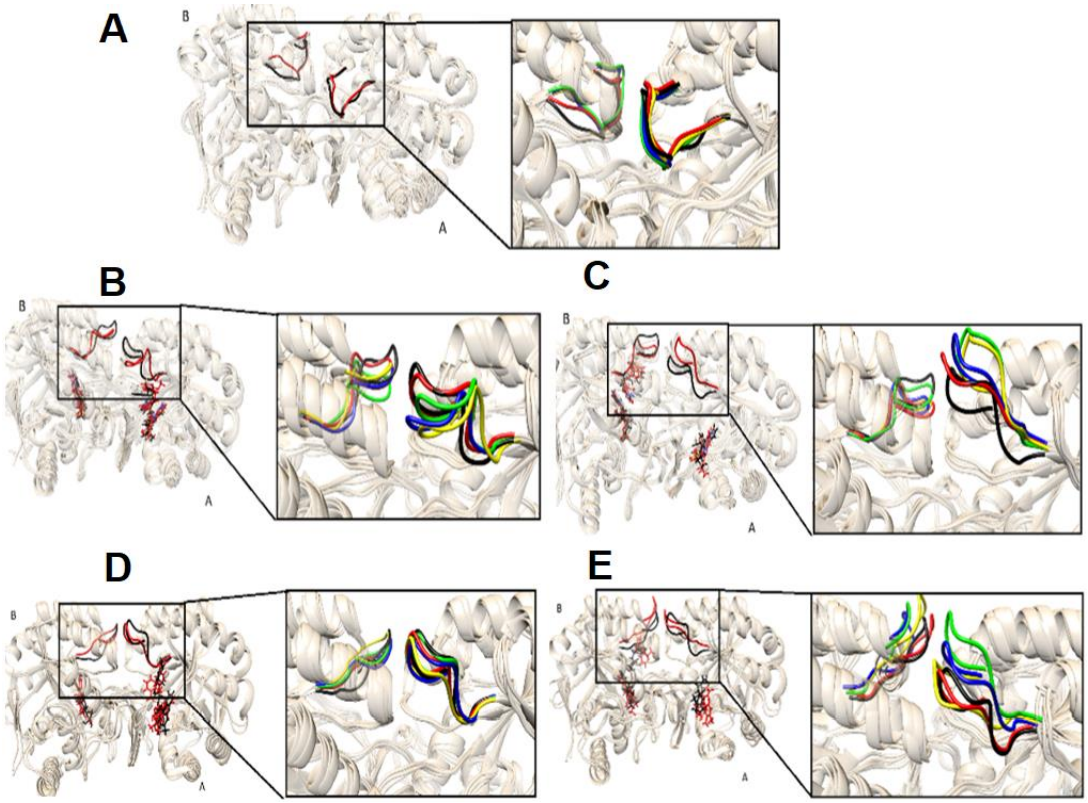
The work was carried out from the following complexes, with PDB code: 3W1X (R = 1.45 Å) [5], ligand XRO (pK_i 5,79 M) and 3W7D (R = 1.52 Å) [5], ligand W7D (pK_i 7,34 M). The molecular dynamic simulations of the protein and its cofactor (flavin mononucleotide) and the ligand complexes were performed in NPT ensemble for 100 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 loop highlighted in Figure 1 is formed by residues Leu128 to Gln138, and seems to be fundamental to the mechanism of action, with alternating opening and closing. The analysis of the structures allowed establishing relationships between the ligands and the catalytic loop. In the holo structure, the loops barely move, the absence of the ligand does not disturb the highlighted region. However, in the complex with the ligand with the highest inhibitory potential (W7D), its presence generated a movement

of the catalytic loop, moving it closer to the ligand, indicating a movement of cavity closing in both trajectories, when bound to the A and B subunits. Still in the SDM of the 3W7D crystal, the catalytic loop of the ligandless subunits had a very accentuated movement, mainly the A subunit, indicating that the presence of a potent ligand in one of the two subunits is capable of perturbing both loops. On the other hand, in the analysis of the 3W1X complex formed with the less potent ligand (XRO), a smaller movement of the loop was observed. A ligand with a lower inhibitory potential does not seem to influence the movement of the loop as much as a ligand with a higher inhibitory potential. Despite the displacement, the final and initial poses were quite similar with the XRO ligand in the B subunit, evidencing that XRO was not able to interfere with the movement of the loops in this simulation.

Figure 1. Active loop movement in the absence and presence of ligands with different inhibitory potentials. The colors in the zoomed image represent the following simulation times: black (0 ns); red (25 ns); green (50ns); blue (75 ns); yellow (100 ns). (A) Holo; (B) W7D subunit A; (C) W7D subunit B; (D) XRO A subunit; (E) XRO subunit B.



Conclusion

This result indicates that the presence of a ligand, regardless of the inhibitory potential, is capable of perturbing the loop. The potency of the inhibitor can be decisive for this perturbation to have an effect on the mechanism of alternation between the open and closed conformational states of the loop, resulting in a loss of enzymatic activity.

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