ANALYSIS OF MOLECULAR DYNAMIC BEHAVIOR OF *Trypanosoma cruzi* DIHYDROOROTATE DEHYDROGENASE COMPLEXED WITH INHIBITORS

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Introduction

Chagas disease represents a serious public health problem, with approximately 8 million people infected worldwide, mainly in Latin American countries where the disease is endemic. There is no effective treatment against this 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 (WHO, 2020). The available treatment consists of the use of two drugs known as benznidazole and nifurtimox, which are mainly used in the acute phase of this disease. Unfortunately, these drugs have low effectiveness in adults with the chronic phase and also show high adverse effects. Due to low activity in the acute and chronic phase of the disease and side effects during treatment, it is necessary to develop new drug candidates. The research and development process for new drugs is considered to be high risk due to the large financial investment required with a relatively low success rate. However, computer-aided drug design (CADD) has been consolidated as an important tool in this process, contributing to cost reduction and increased chance of reaching a new drug. Drug planning using CADD allows for the study of three-dimensional structures of molecules of interest and properties that enable interactions with a target biomacromolecule, usually an enzyme or receptor, leading to a better direction within the research (OU-YANG *et al.,* 2012). Molecular dynamics (MD) simulations is a CADD technique that is able to simulate the behavior of crystallized proteins through computational calculations based on molecular mechanics. In this way, MD simulation is used to plan new drugs and aid in the understanding of the molecular mechanism of proteins (SANT'ANNA, 2002; HOLLINGSWORTH, 2018). Among the possible targets of *T. cruzi*, the role of dihydroorotate dehydrogenase (DHODH) in several metabolic pathways of the parasite makes this enzyme an attractive target for the design of novel anti-*T. cruzi* drugs (REIS *et al.,* 2017). In this work, we have used MD simulation to understanding the behavior of the *T. cruzi* DHODH-inhibitor complex to planning novel inhibitors as potential trypanocidal drugs.

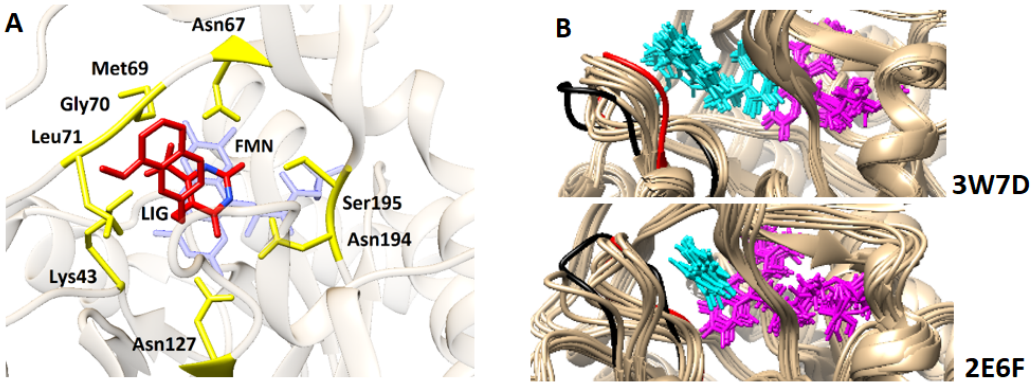
Method

The molecular dynamics (MD) simulations of the protein, cofactor (flavin mononucleotide - FMN), and ligand complexes were performed in the NPT ensemble at a 310 K temperature and at a 1 bar pressure for 50 ns, using the GROMACS 2019.3 software. Molecular structures schematics were presented by Chimera 1.13.1 software and all analyses of MD simulations trajectory were performed using programs present in GROMACS 2019.3 software package. The following *Tc*DHODH-inhibitor complexes, available in the Protein Data Bank - PDB code, were used: 2E6F, Resolution (R) = 1.26 Å (INAOKA *et al.,* 2008), 5E93 (R = 1.41 Å), 3W1A (R = 1.42 Å) (CHELESKI *et al.,* 2010), 3W2J (R = 1.42 Å), 3W87 (R = 1.43 Å), 3W1X (R = 1.45 Å), 3W75 (R = 1.47 Å), 3W23 (R = 1.48 Å), 3W86 (R = 1.5 Å), 4JD4 (R = 1.51 Å) and 3W7D (R = 1.52 Å) (INAOKA *et al.,* 2017).

Results / Discussion

We performed the analysis of hydrogen bonding interactions by molecular dynamics for 11 inhibitor-protein/FMN complexes available in PDB during 50 ns of simulation. The residues Lys43, Asn67, Met69, Gly70, Leu71, Asn127, Asn194, and Ser195 showed higher frequency of interactions and persistence of interactions, with at least five hydrogen bonds with four or more ligands being observed, demonstrating their relevance for the inhibitor-enzyme complex (Figure 1A). The RMSD analysis showed different behaviors between the higher and lower inhibitors, pKi 7.34 (W7D from PDB: 3W7D) and pKi 4.66 (OXC from PDB: 2E6F), respectively. Although both complexes reached their stability early in the simulation, the W7D stabilized with an average RMSD of 1.1 Å and OXC with an average RMSD of 2.4 Å. Also, the structure of the ligands caused conformational variations in the active loop of the protein (Figure 1B), changing the volume of the active site, which is essential for the inhibition of the enzyme.

Figure 1. Analysis of molecular dynamics simulations. (A) Amino acid residues from the DHODH active site that are involved with most of the hydrogen bonds with the ligands. (B) Movement of the active loop along the trajectory, in black the initial position, in red the final position, in cyan the ligand, and magenta the cofactor.



Conclusion

We were able to identify relevant hydrogen bonding interactions in *Tc*DHODH-inhibitor complexes through the molecular dynamics simulations. The data showed a variation on the volume of the cavity of the active site related to the movement of the loop region, among higher and lower active inhibitors. These results may contribute to the construction of a pharmacophore mapping and planning of novel inhibitors.

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