THE DYNAMIC BEHAVIOR OF THE CRUZAIN ENZYME IN ITS FREE FORM AND COMPLEXED TO BENZIMIDAZOLE INHIBITORS

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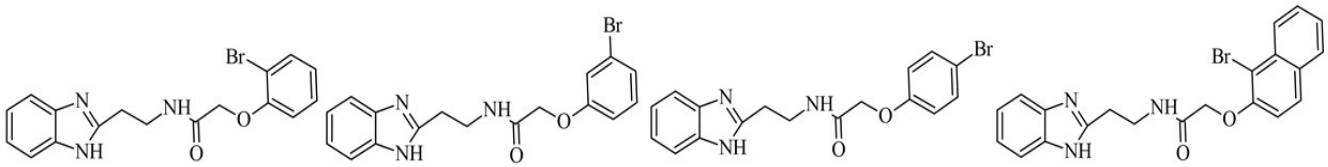
Introduction

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*), presenting alarming rates of infection and mortality. However, there are only two drugs used to treat this disease, nifurtimox, and benznidazole. Although effective in the acute phase of the disease, these drugs are unsatisfactory in the chronic phase, instituting the need for more effective treatment in all phases of Chagas disease (LUCHI *et al*., 2019). Therefore, in the search for new therapies, the enzyme cruzain (CRZ) has been considered a therapeutic target, since its inhibition results in a possible treatment for *T. cruzi* infection in all stages of Chagas disease. Thus, Ferreira and collaborators (2014) identified some CRZ inhibitors of the benzimidazole class with trypanocidal activity. Hence, the scope of this work is to conduct a study at the atomic-molecular level to understand the CRZ inhibition mechanism, based on four inhibitors (Figure 1) selected from the work of Ferreira and collaborators.

Figure 1. Chemical structure of the cruzain inhibitors (BZ1-BZ4) and their respective mean inhibitory concentration (IC_{50}) values.



Method

Setup and Molecular Dynamics Simulations of CRZ_{apo} and CRZ_{1-4} Systems

The complex between CRZ and BZ1 (CRZ_1 system) was extracted from the Protein Data Bank (PDB code: 3KKU) while the complexes formed between BZ2-BZ4 (CRZ_{2-4} systems) were built using the molecular docking technique on the AUTODOCK 4.2 software (MORRIS *et al*., 2009). Thus, the predicted lowest energy complex of each system was selected as the starting structure in the molecular dynamics (MD) simulations. The simulations were performed through the GROMACS 5.1.8 (GMX) software package (HESS *et al*., 2008), using the CHARMM27 force field, the TIP3P water model, and counter ions to neutralize the total charge of the systems. We use the V-resale thermostat to keep the temperature at 310 K and the Parrinello-Rahman barostat to keep the isotropic pressure coupling at 1.0 atm. Electrostatic and Lennard-Jones interactions were considered within a cut-off value of 1.4 nm. The SETTLE algorithm was used to restrict water molecules, and the LINCS algorithm was applied to the restriction of chemical bonds. Finally, the energy minimization step was performed using the steepest descent method. The steps for balancing the system with 100 ps were performed. The time-step value for the integration was defined as 2.0 fs and the systems were simulated during 100 ns in the NpT ensemble, without restrictions on atomic positions. Then, the trajectories obtained were

analyzed to study the dynamic behavior of CRZ in its free form and in the forms complexed with inhibitors, through the GMX and VISUAL MOLECULAR DYNAMICS 1.9.3 (VMD) software (Humphrey *et al*.,1996).

Results / Discussion

The analysis of the root-mean-square deviation (RMSD) of the alpha carbon atoms of the enzyme (CRZ-Cα) has shown that all the systems achieved stability after 10 ns of simulation. The root-mean-square fluctuation (RMSF) analysis of CRZ-Cα presented similar fluctuation in the five systems, except for the loops **L4** (Thr85-Val108) and **L8** (Asn182-Gly192), where BZ1 and BZ2 were able to increase the flexibility of **L4** and **L8**, respectively. Also, the radius of gyration (RG) and secondary structure (SS) analyses showed similar results in all systems, where secondary structure content is maintained during all the simulation.

The hydrogen bond (H-bond) analysis of the CRZ_1 system has reported interactions mainly between BZ1 and Trp26, Ser64, and Asp161 residues. In the CRZ_2 system, BZ2 interacts with Gln19, Gly23, Ser64, and Asp161. Besides, BZ3 showed H-bond interactions with Gln19, Ser64, Asp161, and Trp184 residues. Finally, in the CRZ_4 system, there were H-bond interactions between BZ4 and the residues Gln19, Gly23, Ser64, and Asp161.

In the network map analysis, the CRZ enzyme is described as a set of Cα united by bonds, whose general topology is represented as a network of communities, which are groups of Cα that are densely connected among them and weakly connected with the rest of the network. Thus, in the system CRZapo, the enzyme has presented seven communities. However, in the CRZ_1 system, CRZ has shown nine communities, while, the CRZ_2 and CRZ_3 systems, has presented eight communities. Finally, the CRZ_4 system, which possesses the most active inhibitor, has shown ten communities. Interestingly, in all CRZ-inhibitor systems, there was an increase in the number of communities in the main α-helix of the enzyme. This helix presents Cys25 in its composition, which is one of the catalytic residues of CRZ (Cys25, His162, and Asn182). Thus, it suggests that the complexation of inhibitors BZ1-4 might promote disruption of the helix macro dipole, inhibiting the enzyme activity.

Conclusion

Overall, this study suggests the inhibitors bind to the CRZ through H-bond interactions, mainly by the residues Gln19, Gly23, Trp26, Ser64, Asp161, and Trp184. Moreover, the enzyme does not change its structural composition and compactness, even with the complexation of the inhibitors. However, the network map analyses show that the inhibitors decrease the correlation among CRZ Cα atoms, increasing the number of atomic communities and promoting a disruption in the helix-1 macro dipole, which presents the catalytic residue Cys25. Therefore, the results presented in this work contribute to a better understanding of the inhibition mechanism of the CRZ enzyme, mediated by benzimidazole class inhibitors, and they are helping to plan new therapeutic agents against Chagas disease.

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