Selectivity Model for Drug Design of Cruzain Inhibitors

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Key words: *cysteine proteases, cruzain, falcipain, cathepsins K and L, GRID/GOLPE, PCA/CPCA, Chagas Disease.*

Introduction

Human parasitic diseases are the foremost threat to human health and welfare around the world. Trypanosomiasis is a very serious infestation against which the efficacy of currently available drugs ranges from limited to none. Thus, there is an urgent need for new chemotherapeutic agents[1]. Cysteine proteases (CPs) are relevant to a key biochemical path of the parasite's life-cycle and also to the parasite-host relationship. In this context, the major cysteine protease from *Trypanosoma cruzi*, cruzain, is an attractive target for antitrypanosomal chemotherapy[2]. Nonetheless, this enzyme is also present in other parasites and the human host, thus finding the appropriate selectivity turns out to be a major step to the design new inhibitors of cruzain.

To identify the molecular determinants of selectivity, a comparative study with 4 different types of CPs was performed using the following steps:

1. 3D structures of four cysteine proteases were retrieved from protein data bank: cruzain (1aim, 1ewl, 1ewm, 1ewp, 1ewo, 1f2a, 1f2b, 1f2c, 1f29, 1me3, 2aim, 1me4, 1u9q); falcipain (2ghu); cathepsin L (1ifc); cathepsin K (2r6n, 2aux, 2auz).
2. All structures were aligned and ligands extracted in DeepView/SPDBV program.
3. Molecular interaction fields (MIF) were calculated in GRID program v.22 (grid-spacing of 1 Å) using probes H_2O; C1: sp^2 carbon; N1: hydrogen bond (HB) donor; O: HB acceptor. Principal Component Analysis (PCA) and Consensus PCA (CPCA) were performed using the program GOLPE v.4.5[3].

Results and Discussion

The PCA scoring plot indicates a good distribution and separation of the cysteine proteases (Fig.1), providing evidence that MIFs encode the desired property, enabling comparisons among CPs through CPCA pseudo-fields (Fig. 2).

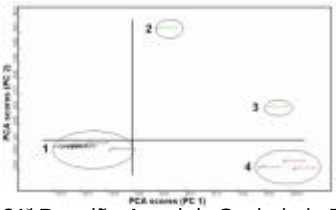

Figure 1. PCA score plot.
1 = cruzain; 2 = falcipain;
3 = cathepsin L;
4 = cathepsin K

The C1 probe shows selectivity spots in the cruzain S2 and S3 pockets, compared to cathepsin L and falcipain, but not cathepsin K (positioning of C1 in S3 changes the selectivity to cathepsin K, Fig. 2A). The H_2O probe showed favorable contributions in the S2 pocket for cruzain over all other CPs, while in S3 cruzain was favored over falcipain and cathepsin L. For the hydrogen bond donor probe (N1) there were selective interactions for cruzain in S1, S3 & S2' (Fig. 2B). The hydrogen bond acceptor (O) probe interacts favorably in the S1 and S3 cruzain pockets.

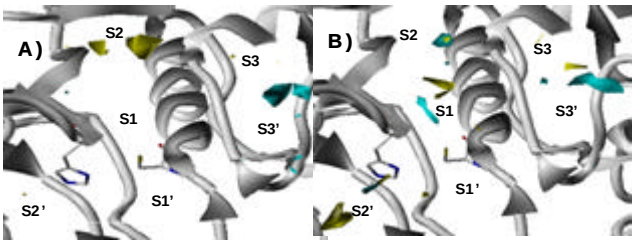

Figure 2. GRID/CPCA pseudo-fields distribution
A) C1 probe; B) N1 probe.
Yellow = favorable to cruzain; Cyan= favorable to cathepisin K.

These results suggest that some hot spots in the protein can be used as guidelines to the virtual screening of putative inhibitors with improved selectivity towards cruzain. A pharmacophore model enclosing this property is under development in order to place key chemical moieties in the right pockets.

Conclusion

Major determinants to cruzain selectivity were observed in the S1 (HB donor and HB acceptor), S2 (lipophilic), S3 (lipophilic and HB donor) and S2' (HB donor) pockets. This model is being applied in the search for new selective cruzain inhibitors.

Acknowledgements

FAPESP (Trossini, GHG, 04/06097-7 PhD Scholarship), and CNPq.

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