Poster I-31

Prediction of functional residues from Plasmodium falciparum plasmepsins: implications in the antimalarial drugs design.

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Short Abstract: Sequence and structural analysis of aspartic proteases allowed us to identify the conserved positions M75, V105, T108, Y115, A219, T221, and L287 (plasmepsin II numbering scheme). The predicted positions are in proximity to the inhibitor's functional groups and they line the protease binding-cavity. This knowledge may contribute to the development of more selective antimalarial drugs.

Long Abstract:

Introduction

Malaria remains one of the world's biggest health problems; 500 million people get infected with the disease each year, and well over one million dies [1]. This infectious disease is caused by parasites from the genus Plasmodium, which survives in certain types of mosquitoes. The microbe is transmitted to humans where it causes many problems, but most commonly severe, recurring fever attacks. The increasing resistance of malarial parasites, in particular Plasmodium falciparum, to the existing antimalarial drugs has focused the research to the discovery of more selective and potent inhibitors. Plasmepsins play vital roles at various stages of the parasite life cycle, and they are attractive targets for antimalarial drug development.

Here, we present a sequence and structural analysis of aspartic proteases including plasmepsins from different Plasmodium species, and their homologous proteins: cathepsins, pepsin, rennin, and napsin. Based on this analysis we predicted seven conserved positions, and their equivalent residues in plasmepsins I, II, III and IV, lining the binding-cavity and close to the inhibitor's functional groups. The positions proposed are different from the active-site residues and have not been studied by site-directed mutagenesis. This knowledge would be useful to develop more selective antimalarial drugs.

Material and Methods

We analyzed 73 amino acid sequences, homologous to Plasmodium falciparum plasmepsin I (PlmI), plasmepsin II (PlmII), histoaspartic protease (HAP), and plasmepsin IV (PlmIV). We also compared 13 crystallographic structures (PDB codes: 1lyw, 1bim, 1f04, 1qdm, 1psn, 1ayf, 1sme, 1qs8, 1ls5, 1fkn, 1lyb, 1xdh, 2bjv) from cathepsin D, pepsin, renin, PlmII, and PlmIV. The following web servers were used: PSI-BLAST (http://www.ncbi.nlm.nih.gov/BLAST) for similarity searches; MC-CE (http://cl.sdsc.edu/) for

structural superposition; CONSURF (http://consurf.tau.ac.il) to calculate the amino acid conservation; GENBEE (http://www.genebee.msu.ru) to calculate and draw the phylogenetic tree; CASTP (http://sts.bioengr-uic.edu/castp) to identify cavities and calculate their area and volumes; WHAT IF (http://swift.cmbi.kun.nl/WIWWWI/) to calculate contacts between residues of the binding-cavity and functional groups of the inhibitors. Multiple alignments were obtained with the CLUSTALW program [2]. Finally, the multiple alignment was parsed by analyzing gaps, conserved amino acid regions and the secondary structure information extracted from the PDB files.

Results and Discussion

P. falciparum plasmepsins has unique substrate specificity, which results due to variations in residues lining the active site cavities [3]. Earlier mutagenesis studies on PlmI and PlmII concluded that differences in substrate-cleavage specificity depend more on conformational differences from distant sites than on specific active site variation [4]. Other authors have studied the regions that undergo structural deviations accompanying ligand binding in PlmI, PlmII, HAP, and PlmIV [5]. The seven conserved positions proposed here (M75, V105, T108, Y115, A219, T221, and L287: PlmII numbering scheme), differ from those previously studied, and are specific to plasmepsins. In addition, we observed that the inhibitor pepstatin A shows low selectivity against human cathepsin D, which could be explained because some of the established contacts in cathepsin D-pepstatin A complex are also present in the PlmII-pepstatin A, and PlmIV-pepstatin A complexes. Nextly, we are going to build models by comparative modelling and docking techniques, for plasmepsins complexes with substrates reported[3], and analize them by molecular dynamic simulations.

References

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