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Structure-based drug designing against Plasmepsins from Plasmodium falciparum

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Plasmodium falciparum is the causative agent of the most lethal form of malaria, killing millions of people annually. Increasing emergence of drug-resistant parasites rapidly necessitates the development of novel and effective anti-malarial inhibitors. A family of ten different plasmepsins are identified in parasite which belong to pepsin-like aspartic protease family. The four food vacuolar plasmepsins (FV PMs), PM I, II, IV and HAP engage in hemoglobin degradation, therefore, are attractive drug targets to fight malaria [1]. Recently, KNI series of inhibitors are found to be very potent against food vacuolar plasmepsins [2].

Among the various KNI inhibitors, evaluated for their inhibitory activity towards PMII, KNI-10333 is found to be extremely potent with a Ki value in sub-nanomolar range. Interestingly, KNI-10743 has shown the highest IC50 value when tested on Plasmodium cultures in our assay. To understand the structural basis of such differences in the Ki values of inhibitors, we have solved the crystal structures of active plasmepsin II in complex with five potent KNI inhibitors (10333, 10343, 10395, 10742 and 10743). The detailed structural analysis of all protein inhibitor complexes have revealed different functional groups in the inhibitors which are important for inhibition. These results suggest that KNI-10333 and KNI-10743 can be used as lead compounds to design effective inhibitors. Based on the structural analysis and kinetics data we have proposed few modifications in the current KNI inhibitors as an approach to develop an inhibitor with increased potency, specificity and good bioavailability.

These plasmepsins are synthesized as inactive zymogens and under acidic conditions, undergo cleavage of their pro-segments to generate active mature enzymes [3]. We have investigated the process of conversion of pro-plasmepsins to mature plasmepsins which is an important step in their maturation. By using Pepstatin A (a potent aspartic protease inhibitor) which binds in the active site of mature enzyme and thus prevents intermolecular activation pathway, our results indicate that zymogen was still capable of activation when pepstatin was present suggesting alternate routes of activation. This activation profile suggests that unlike most other aspartic proteases, initially a simple acid hydrolysis results in formation of initial few mature enzyme molecules of pro-plasmepsin, after which the predominant autocatalytic and trans-catalytic process takes over. To confirm our hypothesis, we have created site-directed mutants of the two catalytic aspartates in PMII, and observed that the activation of the single aspartate zymogen mutants was slow but produced significant level of mature enzyme, which interestingly, have also been shown to degrade hemoglobin. The double mutant D34A/D216A did not undergo processing and thus devoid of any proteolytic activity. Taken together, the study suggests that FV plasmepsins follow different routes for rapid maturation by both active site dependent as well as active site independent pathways. Further insights into the activation process of these proplasmepsins may provide alternative inhibition strategies that could target maturation of plasmepsins.

[1] Coombs, G.H. et al. (2001). Trends Parasitol. 17, 532-537.
[2] Nezami, A. & Freire, E. (2002). Int J Parasitol. 32, 1669-1676.
[3] Tang, J. & Wong, R.N. (1987). J Cell Biochem, 33, 53-63.

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