Histo-aspartic protease (HAP): a unique pepsin-like aspartic protease with catalytic histidine and aspartate

Satadru Chakraborty¹, Subrata Dasgupta¹, Ishan Rathore¹, Vandana Mishra¹, Alla Gustchina², Alexander Wlodawer², Rickey Y. Yada³, Prasenjit Bhaumik¹*

¹Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai-400076, India.  
²Protein Structure Section, Center for Structural Biology, National Cancer Institute, Frederick, MD 21702, USA.  
³Food, Nutrition, and Health Program, Faculty of Land and Food Systems, University of British Columbia, 248-2357 Main Mall Vancouver, BC V6T 1Z4, Vancouver, Canada.

E-mail: pbhaumik@iitb.ac.in

Keywords: Histo-aspartic protease (HAP), Plasmepsin, Catalytic mechanism

Pepsin-like aspartic proteases termed as plasmepsins (PMs) from *Plasmodium* are essential for the different life cycle stages of these parasites, and considered as important therapeutic targets. The deadliest malaria causing parasite *P. falciparum* expresses ten plasmepsins; among which first four PMs (*Pf*PMI, *Pf*PMII, *Pf*PMIII, *Pf*PMIV) are associated with degradation of hemoglobin in the acidic food vacuole of the parasite [1]. Plasmepsins belongs to the classical pepsin-like aspartic protease family which has two catalytic aspartates - Asp34 and Asp214 which are required for the hydrolysis of peptide substrate. The *Pf*PMIII when compared to other members in the family indicates some key substitutions in the active site, most importantly it contains His34 residue in place of the catalytic Asp34 at the N-terminal domain. Due to this substitution, *Pf*PMIII is often referred as Histo-aspartic protease (HAP) [2]. Despite of few recent structural and biochemical studies, the catalytic mechanism of HAP is not understood yet. In this study, we have solved crystal structure of HAP in its Apo-form and identified precise positions of the catalytic as well as surrounding residues. A water molecule is observed close to Asp214 (Fig. 1A); Molecular dynamics (MD) simulations of the substrate bound HAP structure (Fig. 1B) have been performed to understand the dynamic properties of substrate and catalytic residues in HAP active site. Further, the quantum mechanics/molecular mechanics (QM/MM) studies and QM only studies have been used to decipher the catalytic mechanism of HAP. Taking together the results of our studies, we propose a novel mechanism of HAP involving His34 and Asp214 for the catalytic process (Fig. 1C). The enzyme follows general acid/base mechanism for catalysis which is performed in three steps. The initial rate limiting step is associated with generation of gem-diol intermediate. The positively charged His34 is involved in the reaction process as it donates a proton for the formation of the gem-diol intermediate. The second step corresponds to transfer of the proton from the Asp214 to the nitrogen atom of the scissile peptide bond leading to the elongation of the peptide bond length. In the final step, eventually the peptide gets cleaved and formation of products takes place. Both the catalytic residues, His34 and Asp214 return to their initial charged state after the catalysis.

Figure 1. (A) Active site of apo-HAP structure. $2F_o-F_c$ electron density map is shown as purple mesh. (B) Position of the scissile peptide bond in the substrate bound in active site of HAP. The substrate is represented in orange sticks. (C) Schematic representation of the catalytic mechanism of HAP indicating Asp214 and His34 as catalytic base and acid, respectively.
