Poster Presentation

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Understanding the structural basis of legionella pneumophila aminopeptidase N

Anil Kumar Marapaka[1], Anthony Addlagatta[1]
[1]*Centre For Chemical Biology, CSIR-Indian Institute Of Chemical Technology, Hyderabad, India*
E-mail: anilmarapaka@gmail.com

Aminopeptidases catalyze the cleavage of amino acids from the N-terminus of protein or peptide substrates. Aminopeptidase N belongs to M1 family, important for pathogenicity of bacteria and play critical roles in many physiological processes such as protein maturation and regulation of peptide hormone levels in humans. They are widely distributed in animal tissues and found in many subcellular organelles, in the cytoplasm, and as integral membrane proteins [1, 2]. The M1 family is defined by two conserved sequence elements in the catalytic domain; the HEXXH-(X18)-E consensus zinc binding motif and the GXMEN exopeptidase motif [3]. Generally M1 family Aminopeptidases has substrate specificity for positively charged and basic residues at P1 position. Here in our search for genomic database and modeling studies, very interestingly we found a protein Legionella pneumophila Aminopeptidase N with altered signature sequence in M1 family. In this protein MGAMEN exopeptidase motif in ePepN and several other M1-aminopeptidases is replaced by SGASEP and also a Lysine is introduced in the active site pocket where the side chain of N-terminus of substrate binds. From these observations we predict that this enzyme can bind to negatively charged acidic amino acids unlike only the basic and positively charged residues in ePepN and other analogous enzymes. To confirm our predictions we cloned LePepN gene in to pET15b vector, Xho-I and Bam-HI being the restriction sites with N-terminal histidine and transformed into BL21 (DE3) cells. We have optimized expression and purified by affinity fallowed by size exclusion chromatography. We have done biochemical characterization of above purified enzyme, which is supporting our predictions, and determined structure using c and mutational studies are in progress.

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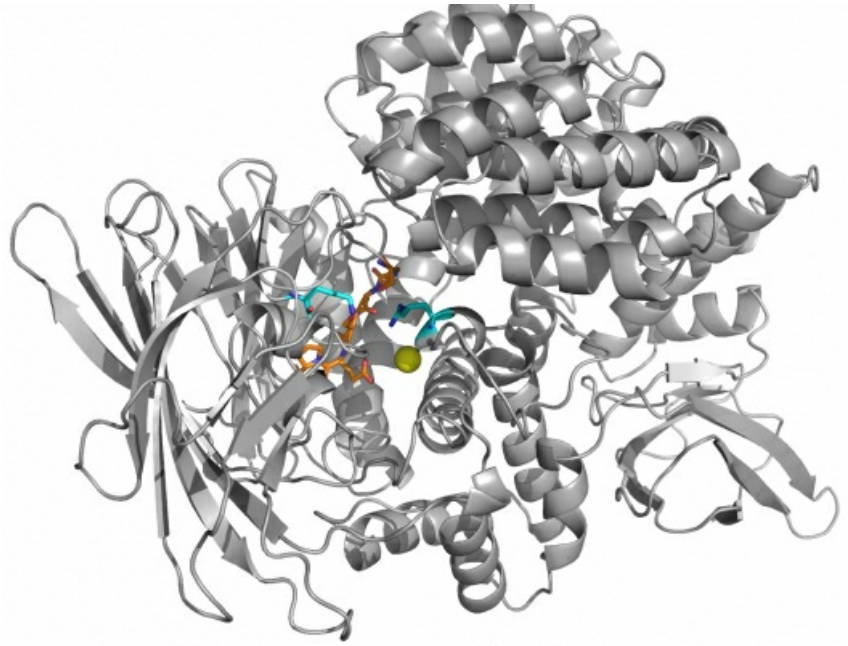

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