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Molecular characterization of ClpP protease from mango chloroplast

*Ishtiaq A. Khan and M. Kamran Azim**
H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

Abstract: ATP-dependent proteasome-like protease systems regulate of many cellular processes by extra-lysosomal protein degradation. Chloroplast genomes of terrestrial plants encode ClpP protease, a proteasome-related ATP-dependent enzyme. We have sequenced the ClpP gene from mango (Mangifera indica) chloroplast. The translated protein sequence was used for structure function studies of this serine protease. Multiple alignment of ClpP sequences from 19 plant species including mango provided an insight of phylogenetic relationships. Mango ClpP protease has grouped with ClpP sequence from *Citrus sinensis* (orange) and *Vitis vinifera* (Grape Vine). Homology model of mango ClpP protease was constructed using crystal structure of *Helicobacter pylori* ClpP as template. The comparison of active sites of bacterial and mango ClpP revealed differences in active site conformation and substrate specificity.

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***Author for Correspondence**: kamran.azim@iccs.edu

INTRODUCTION

Proteolysis requiring metabolic energy is an important part of intracellular protein homeostasis[1]. In eukaryotic cells proteasome is widely recognized as a major macromolecular assembly involved in non-lysosomal, ATP dependent protein degradation, which is responsible for the rapid degradation of many rate limiting enzymes, transcriptional factors and critical regulatory proteins[2]. It is essential for the elimination of abnormal proteins, appearing by means of mutation or by post translational damaged play a primary role in slower degradation of proteins in the bulk of mammalian cells. The proteasome generally degrades target proteins to short peptides, which are further hydrolyzed by the cytoplasmic exopeptidases. However, in higher eukaryotic cells some of these peptides are delivered to the cell surface for the MHC class -I antigen presentation. The 26S proteasome is the main enzyme complex of this protein degradation molecular machine. The proteolytic core of this large complex (~2 mega Dalton) is formed by the 20S proteasome, a barrel shaped structure shown by protein crystallography and electron microscopy to be composed of four rings each containing seven subunits[3]. The 20S proteasomal subunits are classified into groups i.e. and α and β. The seven α subunits comprising the outer rings and seven β –subunits forms the inner ring of the 20S proteasome. In addition to the 20S particles, the 26S complex contains over twenty additional proteins, located in a distinct 19S proteasome complex. The 19S complex determines the substrate specificity and provides multiple ATPase activities necessary for proteolysis.

Several architecturally-related, proteasome like enzyme that require ATP for their function are known[4]. Elegant genomic, biochemical and biophysical studies have provided an array of information regarding ATP dependent protein degradation systems[5-7]. These comprise Lon, ClpAP/ClpXP and HSlvU proteases. The proteases ClpAP/ClpXP consists of two components both of which are required for proteolysis. In ClpAP and ClpXP proteases, the ClpP components confer serine protease activity where as ClpA and ClpX act as ATPase and unfoldase. The ClpA and ClpX proteins which belong to Hsp100 family of ATPase have two and one ATP binding motifs respectively. The protomers of peptidase ClpP organize into two superimposed rings, each having seven subunits, while the protomers of ClpA arrange in a hexameric form in the presence of ATP. Both ATP binding sites in the ClpA appear to be essential for the formation of the ClpP/ClpA complex. The hexameric forms of ClpX ATPase combine with ClpP two-stacked heptameric rings to form an alternative type of ClpP/ClpX protease that is capable of degrading intracellular proteins. Here, we report nucleotide sequence of ClpP protease from mango chloroplast followed by structure function studies of this serine protease.

MATERIALS AND METHODS

Mango chloroplast DNA (cpDNA) isolation

10 gram fresh mango leaves were collected and placed in the dark room at 25˚C for 48 hours to lower the starch level. These leaves were then used to extract DNA. Modified sandbrink buffer (1.25 M NaCl, 50 mM Tris-HCl, 5mM EDTA, 0.1% BSA, 10 mM 2-mercaptoethanol, 4% Polyvinyl pyrrolidone and pH 8.0.) was used for the isolation of chloroplast and ST(0.4 M sucrose, Tris-HCl 50mM and 0.1% BSA) buffer for purification of chloroplast pellet[8-9]. Purified pellet was then treated with DNAse I to remove nuclear DNA contamination. DNA was isolated from purified chloroplast pellet using Multisource genomic DNA minipreprationkit from Axygen Inc.

Sequencing of ClpP gene and sequence analysis

ClpP chloroplast gene was then subjected to DNA sequencing using Genetic Analyzer CEQ8000 (Beckman Coulter Inc. USA). Raw data was analyzed using Trev software of Staden package[10]. SeqMan software of Lasergene DNA analysis package (DNAstar Inc. USA) was used for assembling of analyzed data. The assembled data was then annotated using DOGMA server (Wyman et al. 2004) and translated using ORF finder online tool at http://www.ncbi.nlm.nih.gov/projects/gorf/.

Homology Modeling of ClpP protease

Blast search with mango ClpP sequences against PDB sequences showed alignment of mango sequence with *H. pylori* ClpP with 65% identity. Therefore, the crystal structure of *H. pylori* ClpP was used as template for homology modeling of mango ClpP using protein structure modeling program MODELLER[11] which constructs energy minimized protein models by satisfaction of special restrains extracted from the template PDB files[11]. The input for the MODELLER consisted of aligned sequences of ClpP protein from *H. pylori* and mango.

RESULTS AND DISCUSSION

Plant chloroplast genomes contain the ClpP protease gene. However, the second component of ClpAP or ClpXP protease chaperone complex i.e. ClpA/ClpX ATPase gene is absent in chloroplast DNA (cpDNA). This indicates that the ATPase component is encoded by nuclear genome which then routed towards chloroplast after synthesis in the cytoplasm. Phylogenetic tree grouped mango ClpP protease with *C. sinensis* and *V. vinifera* (Figure 1). Multiple sequence alignment of chloroplast ClpP sequences from 19 species including mango revealed conserved pattern (Figure 2).

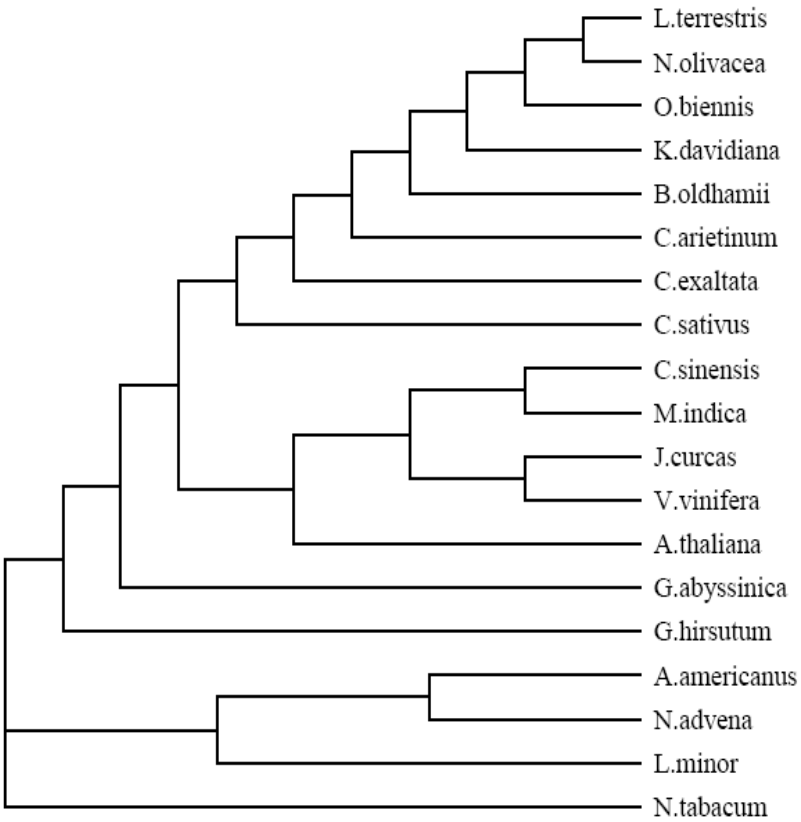

Figure 1: Phylogentic tree of ClpP protein sequences from mango and other 18 plant species.

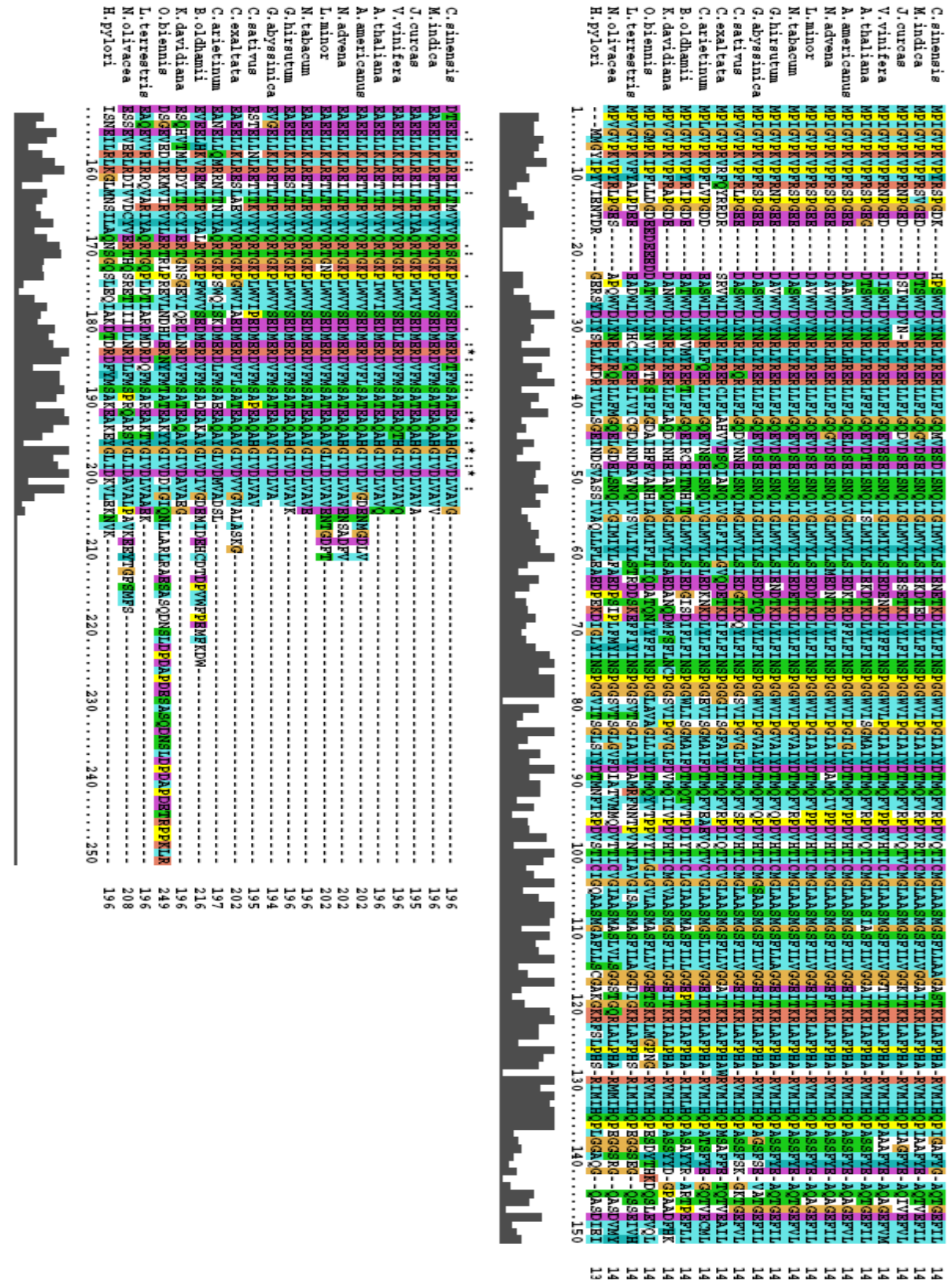

Figure 1: Multiple alignment of ClpP protease sequences from 19 plant species including mango.

Crystal structural coordinates sets of 14 protomeric assebly of ClpP protease from bacteria; Plasmodium falciparum and human mitochondrion are available in PDB. Multiple alignment and structural comparison revealed that the catalytic residues of this serine protease are entirely conserved in chloroplast as well as bacterial and plasmodial ClpP (Figure 1).

Homolgy model of mango clpP provided valuable structural insight (Figure 3). Structural comparison of active site residues of bacterial and mango ClpP indicated that in spite of significant conformational similarity few residues forming certain subsides in the substrate binding cleft are different in mango ClpP. Molecular modeling revealed several conformational variation at the active site regions of bacterial and plant ClpP (Figure 4).

Active site comparison showed that Glu36 and Val71 in bacterial ClpP are substituted with Val37 and Trp71 respectively.

Hence, we conclude that these substitutions would result in distinct subsite and variation in substrate specificity.

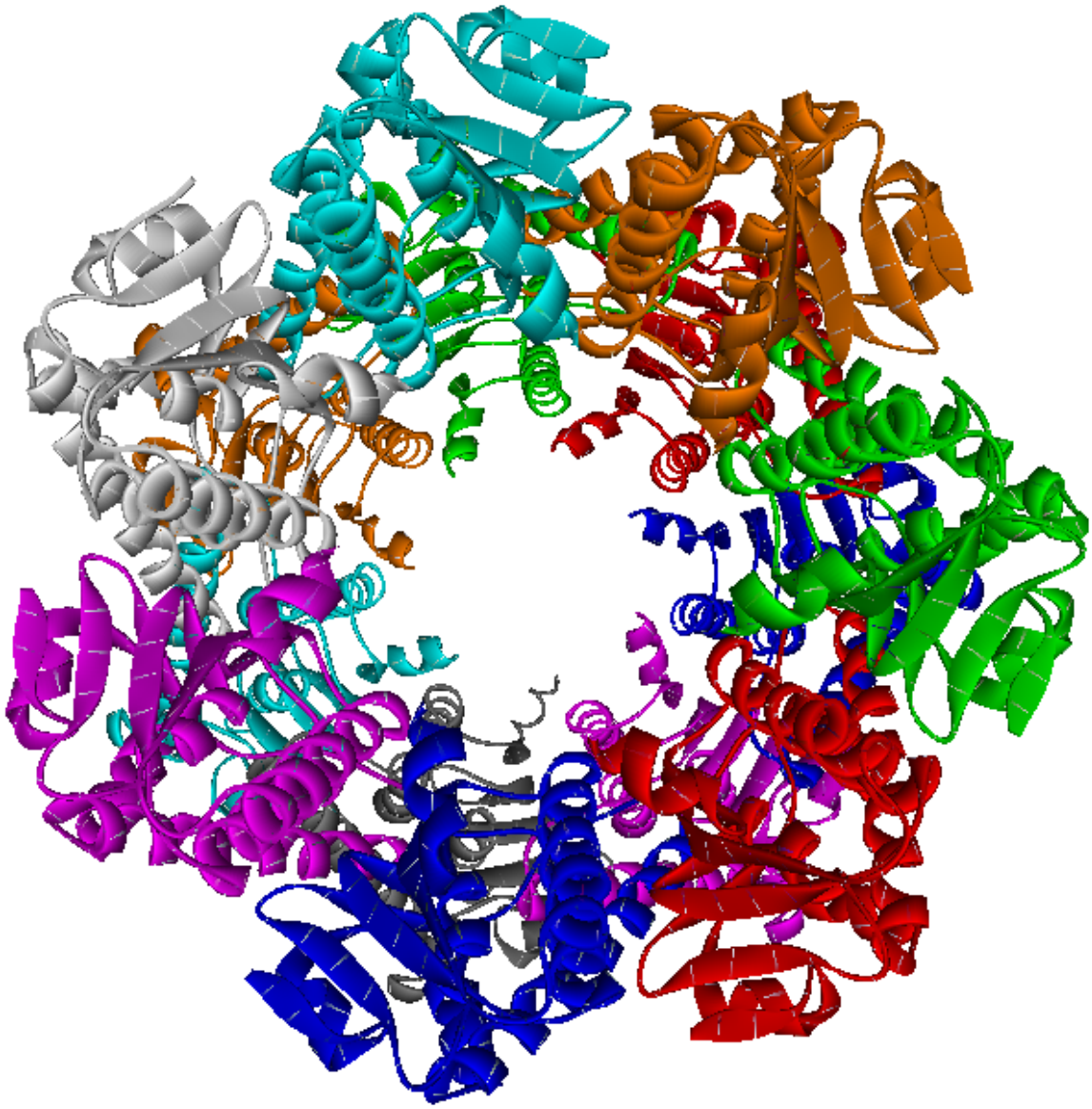

Figure 3: Homology model of the tertiary structure of ClpP protease complex consisting of two heptameric rings (view from top).

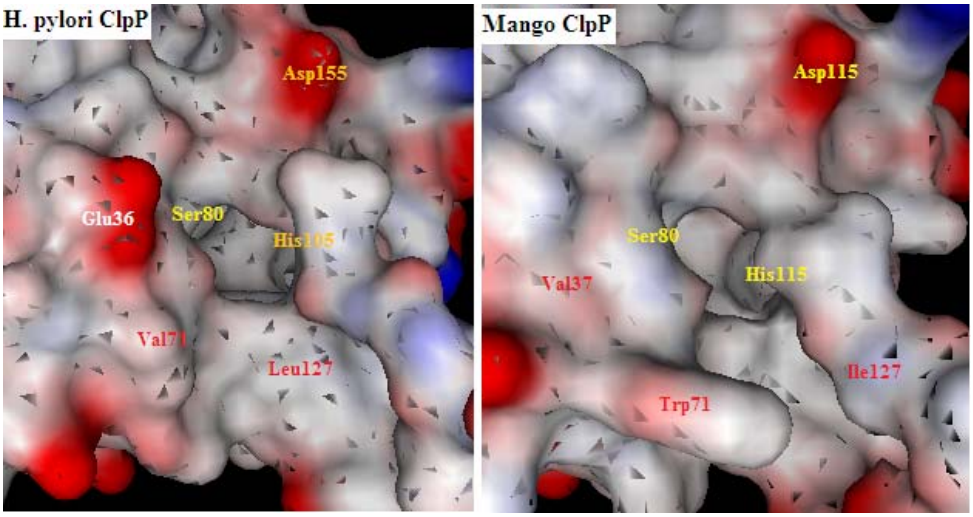

Figure 4: Comparison of electrostatic surface representation of active site regions of ClpP protease from *H. pylori* and mango.

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