

Review

Structure of Prokaryotic HslVU Protease-Chaperone Complex

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Abstract

The HslVU is the prokaryotic, proteasome-related, ATP-dependent protein degrading machinery involved in the proteolysis of short-term regulatory proteins. The HslVU is a two-component system in which the HslV protease that preferentially cleaves after Leu residues is activated by HslU, the ATPase and unfoldase. The subunits of HslU form a hexameric ring where as HslV subunits form a dodecamer of two-stacked hexameric rings. The quaternary structures of HslV and HslU constitute the HslVU complex in which HslV dodecamer forms a central barrel flanked at both ends by HslU hexamers. The HslV and HslU central pores are associated in HslVU complex and the proteolytic sites are sequestered in an internal cavity of HslV dodecamer. During an ATP-dependent process, the HslU hexamer binds, unfolds and translocates the substrate proteins into the proteolytic chamber of HslV. The proteolytic (HslV) and regulatory (HslU) ‘compartments’ of HslVU system occur separately, however, HslU mediates the assembly of the functional complex after it recognizes the native protein substrate.

Energy-dependent protein degradation

Controlled and metabolic energy-dependent protein degradation is a vital element of protein homeostasis inside the cell [1]. In eukaryotes, the proteasome is the central enzyme system for energy-dependent proteolysis. It is accountable for proteolysis of numerous transcriptional factors, critical regulatory proteins and rate-limiting enzymes [2]. The proteasome eliminates anomalous proteins biosynthesized due to gene mutation or by post-translational damage and play a primary role in slower degradation of the majority of proteins particularly in mammalian cells.

The proteasome normally hydrolyzes protein substrates to oligopeptides followed by additional degradation by peptidases in the cytosol. Though, in higher eukaryotes, a few oligopeptides are transported to plasma membrane for the MHC Class-I antigen presentation [1,2]. The 26S proteasome is the central enzyme complex of this proteolytic machinery. The proteolytic staple of this large molecular complex of ~2000 kDa is shaped by the 20S proteasome, a barrel shaped structure

revealed by X ray crystallography and electron microscopy to be comprised of 4 rings of protein subunits each encompassing 7 subunits [3]. These 4 rings of 20S proteasomal subunits are comprised of α - and β - subunits. Architecturally, 7 α -subunits and 7 β -subunits encompass the outer and inner rings respectively. Besides the 20S particle, the 26S proteasome encompasses >20 added proteins, situated in a separate 19S particle. The 19S complex provides substrate specificity and ATPase activities essential for protein turnover.

ATP-dependent proteolytic systems in bacteria

Bacteria generally lack proteasome system, but contain a number of structurally-correlated, proteasome-like enzymes that necessitate ATP for their utility [4]. Bioinformatic, molecular-genetic and spectroscopic analyses revealed different bacterial energy-dependent proteases including Lon, FtsH, ClpAP/ClpXP and HslVU proteases [2,5-7]. Protease Lon, the *lon* gene product, is a heat shock protein, which has been characterized as the homo-oligomer of 87 kDa subunits [8]. Each subunit has an ATP binding site and a Ser

active site for proteolysis, indicating the presence of ATPase and peptidase activities in the same polypeptide chain. The Lon protease quickly degrades misfolded proteins and regulatory proteins including CcdA, RcsA and Sula [9].

Unlike Lon protease, the ClpAP/ClpXP [10] and HslVU proteases (also known as ClpQY) [11] consist of 2 protein-based ‘modules’, together these modules are needed for protein degradation. In the ClpXP and ClpAP, ClpA and ClpX exhibit both unfoldase and ATPase activities; and the ClpP provides serine protease activity [10,12]. The ClpXP complex degrades substrates including λ O protein and the bacteriophage Mu vir repressor [13].

Proteasome-related HslVU protease-chaperone system

The HslVU protease-chaperone system is comprised of 2 heat shock proteins, the HslV (19 kDa) and the HslU (50 kDa). The *hslVU* operon contains genes for these enzymes [11,14]. Structure-function studies identified HslV as an amino-terminal Threonine peptidase allied to β -subunits of 20S proteasomes of archaeobacteria and eukaryotes [15] (Figure 1). Therefore, the bacterial HslV protease has been considered as the evolutionary ancestor of the eukaryotic 20S proteasome [16]. The HslU, like ClpX, is a Hsp100 family member [17] and contains Walker consensus ATP-binding motif. At the same time, the HslU exhibits unfoldase/chaperone and ATPase activities [14]. The HslU has been categorized in the superfamily of AAA-ATPase [18]. Hence, the HslU is correlated to the base part of the 19S proteasome that comprises of several AAA-ATPase proteins. Owing to the structural resemblance of HslU and HslV with 19S and 20S proteasome correspondingly, the HslVU complex is of attention as an archetypal structure for the eukaryotic 26S proteasome [6].

The *E. coli* HslVU degrades short-term proteins including the cell-division inhibitor Sula protein, the heat shock factor σ^{32} and

the transcription activator RcsA [19].

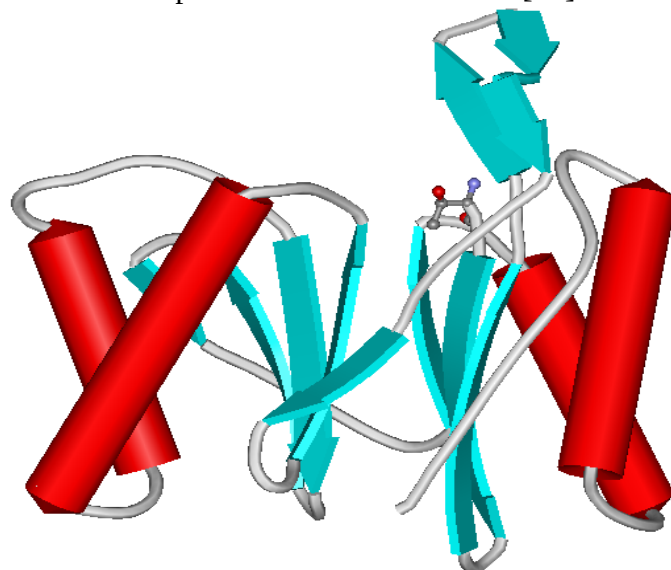


Figure 1: Diagrammatic representation of the crystal structure of *T. maritima* HslV monomer [26]. α -helices and β -sheets are shown by red cylinders and cyan arrows respectively. N-terminal Thr has been shown by ball-and-stick representation.

HslVU also controls the methionine biosynthesis through degradation of enzyme homoserine succinyl transferase [20]. The HslV is expressed on the cell wall of *H. pylori* which indicated involvement of this protease in host-pathogen interaction [21]. *In vitro*, the HslVU degrades oligopeptides along with primarily non-structured proteins including casein [11]. Several observations suggest that HslVU has different substrate specificity compared to archaean proteasome and ClpP protease. With the help of ATP, the HslVU degrades swiftly the fluorogenic substrate Z-Gly-Gly-Leu-AMC and at a slow pace specific ClpP and proteasomal peptides [11]. The cleavage specificity of HslVU toward the natural substrate Sula protein has been determined [22]. The cleavage occurred preferentially after Leu residues of Sula in an ATP-dependent manner. Sula is predominantly degraded by Lon protease [10] and HslVU appears to act as a backup for Lon however, peptide bond

specificities of the two enzymes are distinct from each other [22].

Tertiary structures of HslV and HslU

The crystal structures for the HslV protease of *E. coli* [25], *H. influenzae* [23] and *T. maritima* [26] revealed its similarity with archaean and yeast proteasomal subunits forming a sandwich of two five-stranded anti-parallel sheets, flanked by several helices [27]. The availability of HslV structures from three different sources provided an opportunity to understand the structural basis for the thermo-stability of *T. maritima* HslV (a hyper-thermophilic bacterium; optimum growth temperature= 80°C). Thermal denaturation temperatures (T_m) of *T. maritima* HslV and *E. coli* HslV have been determined by circular dichorism experiments to be >110 °C and 70 °C respectively [26]. Among many structural features, salt bridge interactions are considered to be important determinants of thermo-stability in proteins [28]. The *T. maritima* HslV has been found to have extensive salt bridge network compared to

mesophilic HslV (from *E. coli* and *H. influenzae*). There are 192 salt bridges in the *T. maritima* HslV dodecameric structure exhibiting widespread intra and inter-subunit salt bridge interactions when compared to *E. coli* and *H. influenzae* HslV dodecamers, where the number of salt bridges is 104 and 96, respectively [27]. Comparison of intrasubunit salt bridge interactions between mesophilic and hyper-thermophilic forms of HslV showed extension of four *ion pairs* present in mesophilic HslV into *ion pair clusters* as well as the introduction of two new *ion pair clusters* in thermophilic HslV (Table-I).

The *E. coli* and *H. influenzae* HslU subunits contain three domains, namely nucleotide-binding N-terminal domain (N-domain), carboxy-terminal domain (C-domain) and an intermediate domain (I-domain) [18,23]. The N-domain is topologically related to the N-terminal domain of N-ethylmaleimide-sensitive fusion (NSF) protein whereas I-domain has a loosely folded structure with coiled helices.

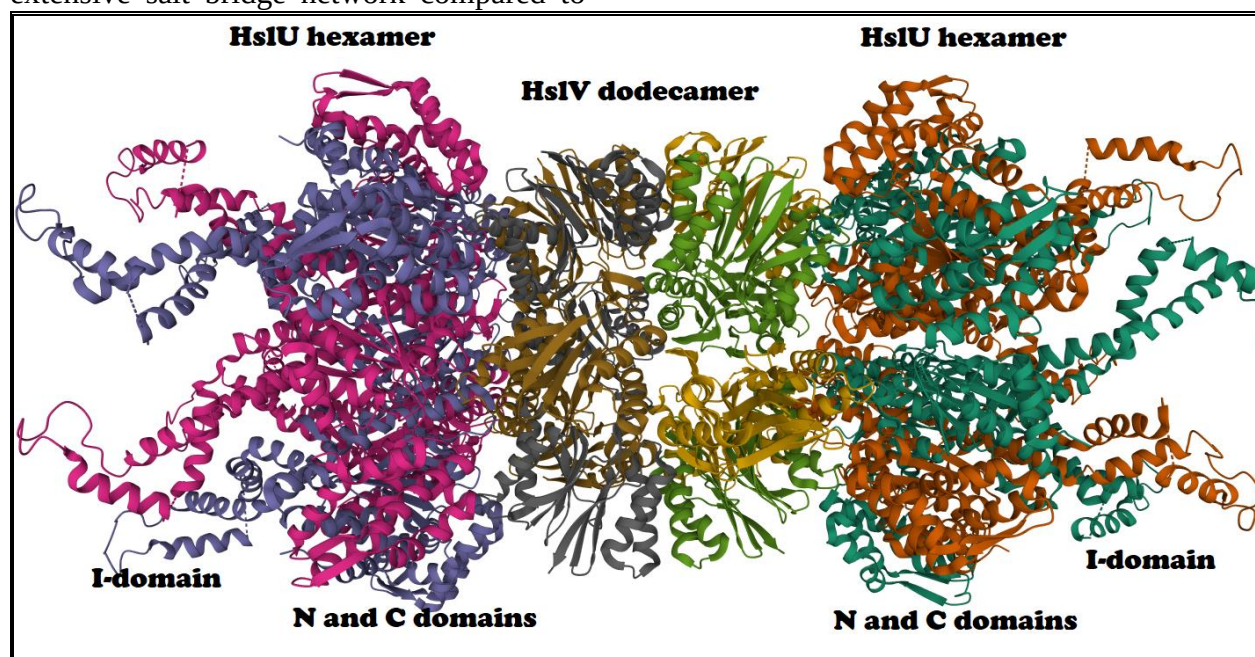


Figure 2: The schematic representation of crystal structure of HslVU complex (PDB ID 1G4B). This structure shows the docking style of HslU to HslV with the I-domains distal to the HslV dodecamer [23,24,29]. The HslVU complex in another crystal structure show complexing of HslU to HslV with I-domain directing to the HslV [18].

Table-I: Comparison of salt-bridge interactions between mesophilic (*E. coli* and *H. influenzae*) and thermophilic (*T. maritima*) forms of HslV protease. One-letter codes for amino acids are used. Ion pairs 8-10 are present in *T. maritima* enzyme only whereas ion pairs 3-5 has extended into salt-bridge networks.

No.	<i>E. coli</i>	<i>H. influenzae</i>	<i>T. maritima</i>
1	R7-D118	-	R7-D118
2	D17-K33	D17-K33	D17-K33
3	R36-E169	R36-E170	K36-E169-R32
4	D81-K63	D81-K63	D85-R59-D81-K63-E66
5	E77-K80	-	K73-E77-K80
6	E149-H166-D153	-	-
7	D157-H164	-	-
8	-	-	E61-R65
9	-	-	K134-D117-R138
10	-	-	E170-R145-E149

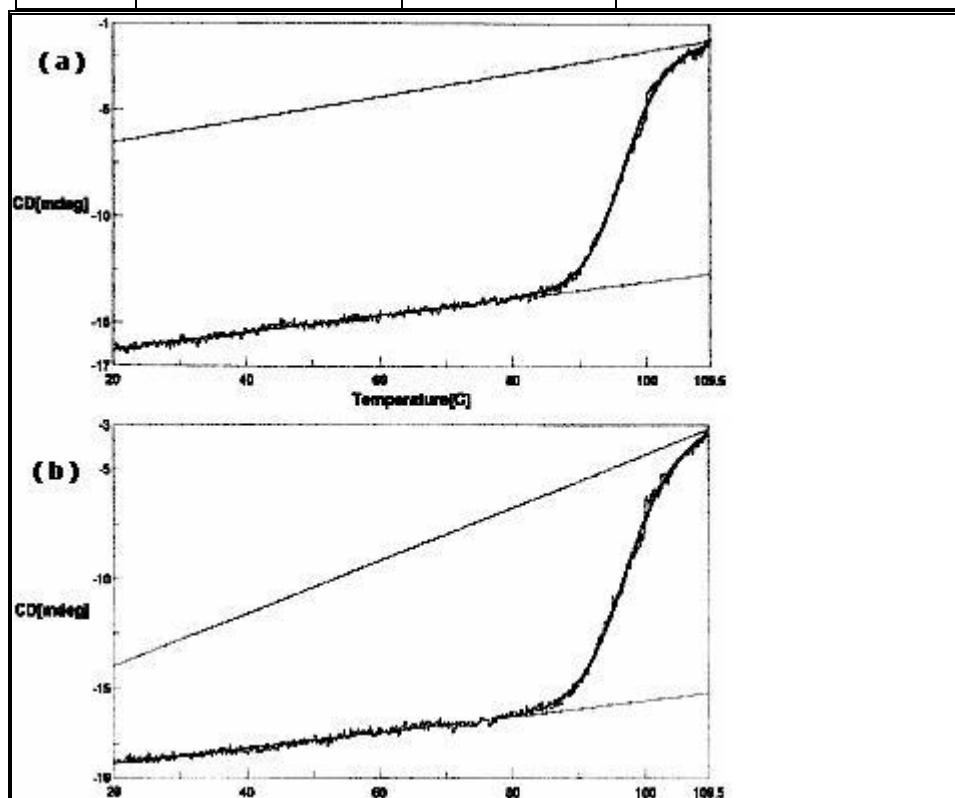


Figure 3: Circular Dichroism spectra of *T. maritima* (a) HslU and (b) HslVU dissolved in Tris buffer containing 5 mM ATP. Ranges of denaturation for HslU and HslVU are 82-106 and 83-108°C respectively. Melting temperatures (T_m) for both samples has been calculated as 96°C.

The intermediate domain is required for recognition and binding of substrate proteins [18]. Thermal denaturation temperatures (T_m) of HslU and HslVU complex of *T. maritima* have been determined by circular dichroism to be 96 °C (Figure 3) [38].

Structural design of HslVU complex

The HslU and HslV form a functional complex when the HslV monomers assemble into a dodecamer of two-stacked hexameric rings and two HslU hexamers bind to the opposite ends of the HslV dodecamer (Figure 2) [18,23,24]. The catalytic clefts of HslV are confiscated in the internal compartment of the doughnut-shaped dodecameric rings of HslV, with entrance to this compartment limited to small axial apertures. The diameters of these apertures are small enough that only unfolded polypeptides can be squeezed through. Consequently, natural folded proteins need to be unfolded by an associated chaperone/unfoldase (i.e. HslU) and translocated into the proteolytic chamber [18,23]. This process entails particular docking of the unfoldase (HslU) with the peptidase (HslV). During an ATP-dependent process, the HslU hexamer recognizes, unfolds and translocates substrate proteins into the proteolytic chamber of the HslV dodecamer for hydrolysis [6]. Moreover, interaction of HslU stimulates the protease activity of HslV [11].

Docking mode of HslU with HslV

The electron microscopy and X-ray analysis of *E. coli* HslVU complex showed different binding modes of HslU hexamers to HslV dodecamer (Figure 2) i.e. (a) the HslU interfaced to HslV with the I-domain pointing toward HslV particle [18,29] and (b) HslU docks with the HslV with I-domain distal to HslV [23,29]. The *H. influenzae* HslVU complex supported the orientation of HslU and appears similar to the second

binding mode of *E. coli* structure [23,24]. In hexameric *E. coli* HslU structure, seven amino acid residues at C-termini was hidden in the HslU protomer [18]. Whereas in *H. influenzae* HslVU complex, the HslU C-termini inflates and inserts into a cleft flanked by neighboring HslV protomers [23]. This intercalation was complemented by a conformational transformation in the catalytic cleft of HslV. This phenomenon indicated an allosteric activation of HslV by the C-terminal amino acid residues of HslU [23,24]. Furthermore, HslV activation by synthetic peptides containing the HslU C-terminal residues maintained the activity of this region of HslU in the HslVU complex formation and allosteric activation [30].

Assembly of the active HslVU complex

Binding of HslV to HslU results in a moderately labile HslVU complex which does not survive most chromatographic procedures [6,11,18]. It is therefore easy to purify both components separately. This type of behavior has also been observed in ClpAP protease complex, which dissociates into its components during purification procedures [32]. Hence, need of supplementary proteins/molecules for the stability and formation of fully functional HslVU chaperone-protease was expected [11]. *In vivo* studies using yeast two-hybrid system also revealed that *E. coli* HslV and HslU have a fragile association [33]. ATP and carboxy terminal tails of HslU have been recognized as factors which play a role in HslVU complex formation [30,31,34]. Binding assays using Surface Plasmon Resonance (SPR) technology revealed that affinity of HslU increases for HslV considerably followed by binding with Sula protein signifying construction of a 'ternary complex' [35]. On the basis of protein-protein interaction studies of HslVU and its protein substrates, a model for the catalytic cycle of HslVU has proposed (Figure 4) [35].

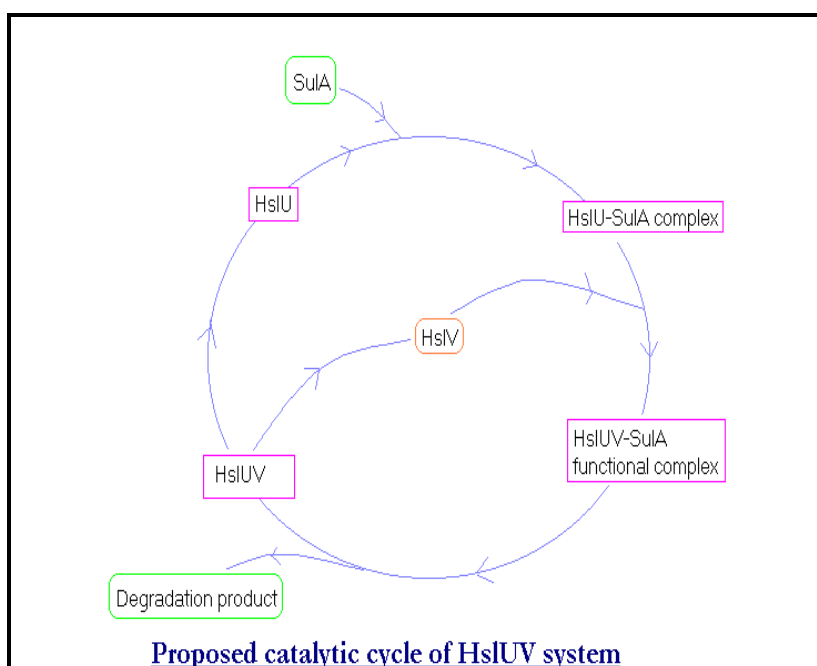


Figure 4: Molecular mechanism during the proteolytic cycle of HslVU system. The HslU shuttles amid states of low and high affinity for HslV. The affinity of HslU increases after binding with protein substrates such as SulA. After proteolysis, the HslVU dissociates into HslV dodecamer and HslU hexamer(s) [35].

This model can be applied to similar ATP-dependent proteolytic systems such as ClpAP/ClpXP [36]. According to this model, two components of HslVU protease exist separately in solution. The regulatory component HslU with its I-domain recognizes and binds the folded substrates destined to be degraded, hence, the HslU-substrate complex is generated. The binding to HslU requires specific sequences in protein substrates such as the carboxy-terminal eight residues of SulA have been described as recognition site for Lon protease [37]. Most likely, HslU also utilizes the same mechanism for SulA recognition. Following events activate conformational changes in the HslU structure which would result in significant increment of its affinity for HslV and a functional complex of HslVU-substrate is assembled [31,35]. The HslU carboxy-terminus plays an important role in HslVU complex formation, therefore, presumably substrate unfolding and/or translocation cause the buried carboxy-terminal peptides to distend and intercalate in between adjacent subunits of the HslV

dodecamer [24]. Likewise, during the later stage(s) of HslVU activity when the degradation products are released, the carboxy-terminal peptides detach from the HslV dodecamer which lowers the affinity of two components for each other.

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