Mol. Cells, Vol. 23, No. 2, pp. 252-257

Molecules and Cells

Nucleotide Triphosphates Inhibit the Degradation of Unfolded Proteins by HslV Peptidase

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(Received January 23, 2007; Accepted January 29, 2007)

***Escherichia coli* HslVU is an ATP-dependent protease consisting of two heat shock proteins, the HslU ATPase and HslV peptidase. In the reconstituted enzyme, HslU stimulates the proteolytic activity of HslV by one to two orders of magnitude, while HslV increases the rate of ATP hydrolysis by HslU several-fold. Here we show that HslV alone can efficiently degrade certain unfolded proteins, such as unfolded lactalbumin and lysozyme prepared by complete reduction of disulfide bonds, but not their native forms. Furthermore, HslV alone cleaved a lactalbumin fragment sandwiched by two thioredoxin molecules, indicating that it can hydrolyze the internal peptide bonds of lactalbumin. Surprisingly, ATP inhibited the degradation of unfolded proteins by HslV. This inhibitory effect of ATP was markedly diminished by substitution of the Arg86 residue located in the apical pore of HslV with Gly, suggesting that interaction of ATP with the Arg residue blocks access of unfolded proteins to the proteolytic chamber of HslV. These results suggest that uncomplexed HslV is inactive under normal conditions, but may can degrade unfolded proteins when the ATP level is low, as it is during carbon starvation.**

Keywords: ATP-dependent Protease; ATPase; HslU; HslV; Lactalbumin; Unfolded Proteins.

Introduction

ATP-dependent proteolysis plays an essential role in eliminating proteins with aberrant structures and in controlling the levels of key regulatory proteins (Goldberg, 1992; Gottesman and Maurizi, 1992). In bacteria, these functions are carried out by ATP-dependent proteases, such as Lon (protease La), ClpAP (protease Ti), ClpXP, HslVU (ClpQY), and FtsH (Chung, 1993; 1997; Gottesman, 1996; 2003; Maurizi, 1992; Park *et al*., 2006). HslVU is the product of the *hslVU* operon, which encodes two heat shock proteins, the HslV peptidase and the HslU ATPase (Chaung *et al.*, 1993). HslV forms a dodecamer of two back-to-back stacked hexameric rings (Bochtler *et al.*, 1997) while HslU is a hexameric ring that binds to either one or both ends of HslV to form the HslVU complex (Bochtler *et al.*, 2000; Wang *et al*., 2001a). In the HslVU complex, the central pores of HslU and HslV are aligned and the proteolytic active sites are sequestered in the internal chamber of HslV, with access to this chamber restricted to small axial pores.

HslV by itself is a weak peptidase that slowly degrades some hydrophobic peptides, such as N-carbobenzoxy-Gly-Gly-Leu-7-amido-4-methylcoumarin (Z-GGL-AMC), and certain unfolded proteins, like casein and insulin B-chain (Rohrwild *et al*., 1996; Yoo *et al*., 1996). In the presence of ATP, HslU markedly stimulates the peptide- and protein-degrading activity of HslV (up to about 100-fold) by forming the HslVU complex. Interestingly, ATPγS, a non-hydrolyzable ATP analog, supports the hydrolysis of the insulin B-chain and Z-GGL-AMC by HslVU even more effectively than ATP. However, ATPγS and other ATP analogs cannot support the degradation of native, folded protein substrates, such as SulA, an inhibitor of cell division (Seong *et al.*, 1999). Thus, ATP hydrolysis by HslU ap-

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pears to play an essential role in unfolding native protein substrates for subsequent translocation into the inner proteolytic chamber of the HslV dodecamer. In addition, HslU alone can function as a molecular chaperone preventing aggregation of SulA, which has an intrinsic tendency to form aggregates under both *in vitro* and *in vivo* conditions, although ATP hydrolysis appears not to be essential for its chaperone function (Seong *et al.*, 2000).

α-Lactalbumin (LA), which has four disulfide-bonds and binds a single Ca^{2+} ion, is one of the model proteins studied most extensively to clarify mechanisms of protein stabilization, folding, and unfolding. Under a variety of mild denaturing conditions, such as dissociation of Ca^{2+} or partial reduction of disulfide bonds, LA adopts a partially structured conformation termed a 'molten globule' that is intermediate between native and unfolded states (Acharya *et al*., 1991; Hiraoka *et al*., 1980). Fully reduced LA no longer has a native conformation with little or no detectable secondary structure.

In an attempt to define the degree of unfolding required for degradation by HslVU, we used LA as a model protein in various unfolded states as substrate. In the present study, we found that HslV can by itself degrade unfolded LA (u-LA), but not the native form or partially unfolded intermediates. More surprisingly, ATP was found to inhibit the activity of HslV by interacting with the basic apical pore region of the HslV dodecamer. Possible roles of ATP and uncomplexed HslV in the degradation of unfolded proteins are discussed.

Materials and Methods

Materials Calcium-depleted bovine α-lactalbumin (LA), chicken egg white lysozyme, and bovine pancreatic RNase A were purchased from Sigma. Z-GGL-AMC was obtained from BACHEM Feinchemikalien AG (Switzerland). A peptide corresponding to the C-terminal ten amino acids of HslU (NH_2-ADEDLSRFIL-COOH) was synthesized by AnyGen, Korea. Plasmid pBS/HslV that expresses HslV only was constructed as described (Yoo *et al*., 1997a).

HslV and HslU were purified as described previously (Yoo *et al*., 1997b). Partially and fully reduced forms of LA were prepared as described (Ewbank and Creighton, 1993). Fully reduced forms of lysozyme and RNase A (10 mg/ml) were prepared by treatment with 20 mM DTT in the presence of 8 M urea. Protein concentrations were estimated according to Bradford using bovine serum albumin as standard (Bradford, 1976).

Assay of proteolysis Degradation of proteins was assayed as described previously (Seong *et al*., 2002). Reaction mixtures (0.1 ml) contained protein substrates (7 nM) and HslV (50 nM) in 25 mM Tris-HCl (pH 8), 2 mM DTT, and 0.5 mM EDTA in the absence or presence of 1 mM adenine nucleotides. To assay degradation of the reduced forms of proteins, 2 mM DTT was added to the reaction mixtures. After incubation at 37°C, the reactions were stopped by adding 30 μl of 0.75 M Tris-HCl (pH 6.8) containing 7.5% (w/v) SDS and 10% (v/v) 2-mercaptoethanol. The mixtures were then subjected to Tricine-SDS-PAGE as described (Schagger and Jagow, 1987). After staining the gels with Coomassie blue R-250, densitometry was performed to estimate the amounts of substrate remaining using a LAS-3000 and MultiGauge software (FUJIFILM, Japan), with 5-point standard curve calibration. Peptide hydrolysis was assayed by incubating reaction mixtures (0.1 ml) containing Z-GGL-AMC (0.1 mM) and 20 nM HslV in 25 mM Tris-HCl (pH 8), 1 mM DTT, and 0.5 mM EDTA. Release of AMC was measured by continuous (2-min interval) monitoring of fluorescence using a FLUOstar OPTIMA fluorometer (BMG LABTECH, Germany) (Park *et al*., 2006).

Purification of TLT A cDNA encoding a bovine LA fragment (amino acids 47–111) flanked by two thioredoxin molecules (TLT) was cloned into pET32a, and transformed into strain BL21(DE3)-*hslVU*. The cells were grown in Luria broth to an OD_{600} of 0.6 and induced by treatment with IPTG. TLT was then purified using a Ni^{2+}-NTA column according to the manufacturer's instructions.

Results

HslV by itself degrades unfolded LA In an attempt to define the degree of unfolding required for degradation by HslVU, we used lactalbumin (LA) in various unfolded states as a model substrate. Surprisingly, HslV alone (*i.e*., in the absence of HslU and ATP) degraded fully unfolded LA (u-LA) (Fig. 1A, left panel), but not native LA or partially unfolded intermediates (data not shown). To test whether HslV degraded other unfolded proteins, lysozyme and RNase A, which possess four disulfide bonds like LA, were fully unfolded by reduction of all the disulfide bonds. We also tested whether HslV hydrolyzed casein, a natively unfolded protein (Holt and Sawyer, 1988; Syme *et al*., 2002). HslV degraded u-lysozyme nearly as well as u-LA, but cleaved α-casein at a much slower rate (Fig. 1B, left panel). In contrast, it had no activity against u-RNase A. These result indicate that HslV alone is capable of degrading some unfolded proteins, though at different rates.

The C-terminal tails of HslU play a critical role in the interaction with, and activation of, HslV peptidase (Seong *et al*., 2002). Moreover, a synthetic tail peptide of 10 amino acids (referred to as the C10-peptide) substituted for HslU in promoting HslV-mediated hydrolysis of casein and the insulin B-chain as well as of Z-GGL-AMC. In addition, X-ray crystallographic studies of the structure of the HslVU complex have shown that the highly conserved HslU C-terminus is inserted at the HslV-HslV subunit interface when ATP is bound, but otherwise is buried

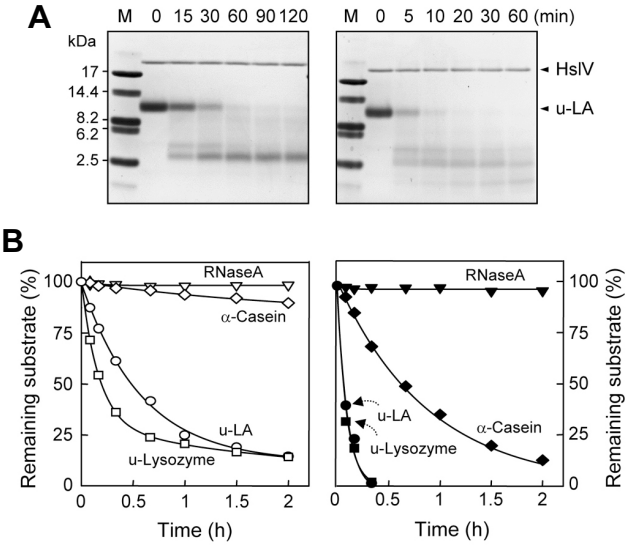

Fig. 1. Degradation of various unfolded proteins by HslV. **A.** HslV (50 nM) was incubated with u-LA (7 μM) at 37°C in the absence (left panel) or presence of 60 μM C10-peptide (right panel). After incubation, samples were subjected to SDS-PAGE followed by staining with Coomassie blue R-250. **B.** HslV was incubated as above but with various unfolded proteins (7 μM) in the absence (left panel) or presence of 60 μM C10-peptide (right panel). The samples were then subjected to SDS-PAGE followed by staining with Coomassie blue R-250. The protein bands were scanned with a densitometer to estimate the amounts of remaining substrate.

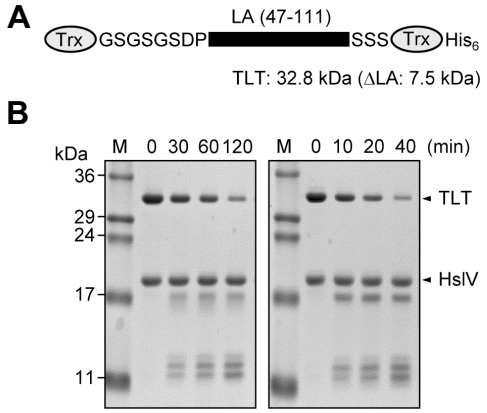

Fig. 2. Cleavage of internal peptide bonds in the LA fragment by HslV. **A.** A hybrid protein comprising two thioredoxin molecules flanked by the amino acid sequence of LA (47–111) was purified as described in **Materials and Methods**. The sizes of the fusion protein (TLT) and the LA fragment (ΔLA) are shown. **B.** HslV (50 nM) was incubated with TLT (1 μM) at 37°C for the indicated periods in the absence (left panel) or presence of 60 μM C10-peptide (right panel). After incubation, the samples were subjected to SDS-PAGE followed by staining with Coomassie blue R-250. Note that the incubations were carried out for shorter times when the C10-peptide was present.

at the HslU-HslU subunit interface (Sousa *et al*., 2000; Wang *et al*., 2001b). Therefore, we have suggested that insertion of the C10-peptide into pockets at the HslV-HslV interface causes a widening of the central pore of HslV peptidase that permits access of peptides or unfolded polypeptide substrates into the proteolytic chamber, thus facilitating their degradation (Seong *et al*., 2002). As expected, incubation of HslV with C10-peptide also markedly increased the rate of hydrolysis of u-LA, u-lysozyme, and α-casein, but not that of u-RNase A (Figs. 1A and 1B, right panels). Thus, it seems clear that some unfolded proteins have access to the inner chamber of the HslV dodecamer and are thus degraded without the aid of the HslU ATPase.

HslV on its own cleaves internal peptide bonds of LA The diameter of the central pore of HslV is so small that only a single strand of polypeptide can be threaded through it (Bochtler *et al*., 1997; Wang *et al*., 1998). This finding is in accord with our observation that HslV alone can degrade fully unfolded LA but not nascent or partially unfolded intermediates. To confirm this, we constructed a hybrid protein, with a fragment of LA (amino acids 47–111) sandwiched by two thioredoxin molecules (Fig. 2A). HslV alone could also degrade this hybrid protein (Fig. 2B, left panel) and its proteolysis was markedly stimulated by C10-peptide (right panel). However, thioredoxin was not degraded by HslV (data not shown).

ATP inhibits HslV-mediated degradation of u-LA and Z-GGL-AMC To determine whether HslU in the presence of ATP facilitates the HslV-mediated degradation of u-LA, HslV was incubated with HslU, ATP, or both. In fact HslU in the presence of ATP inhibited the degradation of u-LA by HslV (Fig. 3A) while HslU in the absence of ATP had little or no effect on HslV activity on u-LA. To confirm the inhibitory effect of ATP, HslV was incubated with u-LA in the presence of increasing concentration of ATP. Figure 3B shows that ATP inhibited HslV-mediated degradation of u-LA in a concentration-dependent fashion. Similar inhibitory effects were observed with α-casein and u-lysozyme as substrates (data not shown). We conclude that ATP, not HslU, is responsible for the inhibition of HslV. We next examined whether other adenine nucleotides also inhibited HslV-mediated hydrolysis of u-LA and Z-GGL-AMC. While AMP had little or no effect, ADP did inhibit HslV activity but only about 30% as well as ATP (Fig. 3C). Similar results were obtained for peptide hydrolysis (Fig. 3D). Inorganic pyrophosphate inhibited HslV-mediated degradation of u-LA nearly as well as ADP, whereas inorganic phosphate had little or no effect. In addition, other nucleotide triphosphates inhibited HslV activity to a similar extent as ATP (data not shown). The inhibitory effects of nucleotide triphosphates was not re-

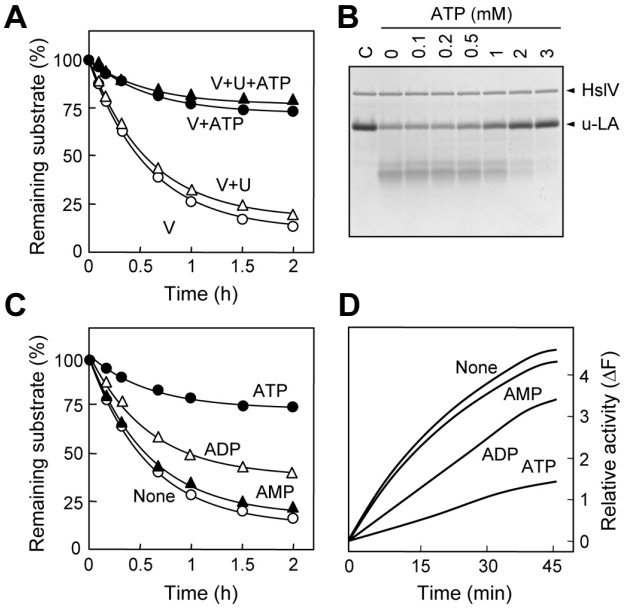

Fig. 3. Effects of adenine nucleotides and HslU on the hydrolysis of u-LA and Z-GGL-AMC by HslV. **A.** HslV (50 nM) was incubated with u-LA (7 μM) at 37°C for increasing periods in the presence of HslU (10 nM), ATP (1 mM), or both. The letters V and U denotes HslV and HslU, respectively. **B.** HslV (50 nM) was incubated with u-LA (7 μM) at 37°C for 1 h in the presence of increasing concentrations of ATP. **C.** HslV (50 nM) was incubated with u-LA (7 μM) at 37°C for increasing periods in the presence of the indicated adenine nucleotides (1 mM). **D.** HslV (20 nM) was incubated with 0.1 mM Z-GGL-AMC and 20 μM C10-peptide at 37°C for increasing periods in the presence of the indicated adenine nucleotides (1 mM). Peptide hydrolysis was then assayed as described in **Materials and Methods**.

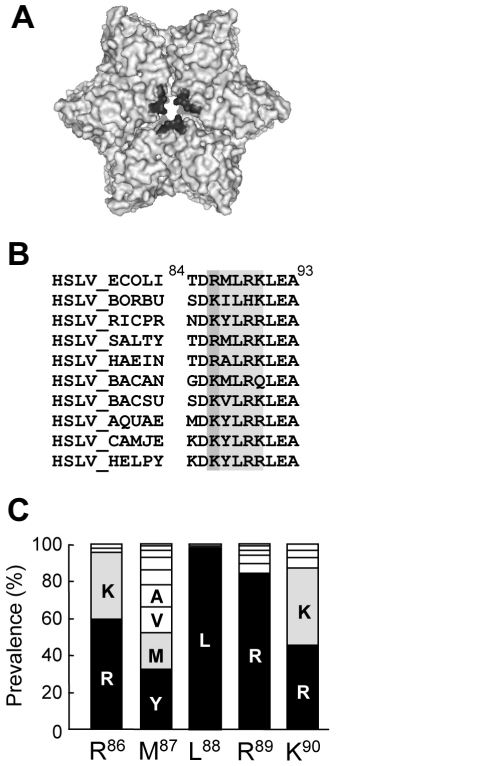

Fig. 4. Structure of HslV and sequence of the apical pore region. **A.** X-ray crystal structure of an HslV dodecamer (Protein Data Bank code: 1HT1) viewed from the side proximal to HslU. The central dark region shows the Arg86 residue in the apical pore region of HslV. **B.** Alignment of the amino acid sequences of the HslV pore motifs of various micro-organisms. **C.** Prevalence of each amino acid in the HslV pore motif shown as a percentage. The data were obtained from 93 reference sequences.

lieved by high concentrations of NaCl (*e.g*., 0.2 M), suggesting that the inhibition is not due to a simple charge effect.

Effect of the R86G mutation on HslV activity Structural analysis has shown that basic amino acids are clustered in the apical pore region of the HslV dodecamer (Fig. 4A). Moreover, database analysis revealed that the basic pore motif (amino acids 86–90) is highly conserved (Fig. 4B) and that more than 95% of the HslVs in 93 reference sequences have either Arg or Lys at position 86 (Fig. 4C). To see whether the inhibitory effect of ATP on the HslV-mediated degradation of u-LA and Z-GGL-AMC is due to the interaction of ATP with Arg86 we replaced the Arg residue of *E. coli* HslV with Gly by site-directed mutagenesis. The R86G mutation markedly reduced the ability of HslV to degrade u-LA and other unfolded proteins (data not shown). In contrast, it hydrolyzed Z-GGL-AMC nearly as well as wild-type HslV (Fig. 5A), indicating that the mutation does not alter the catalytic activity of HslV. Furthermore, the inhibitory effect of ATP on peptide hydrolysis was dramatically reversed by the R86G mutation (Fig. 5B). These results strongly suggest that binding of ATP to the basic apical pore region, in particular to Arg86, prevents access of unfolded proteins and peptides to the proteolytic core of the HslV dodecamer.

Discussion

The present study has demonstrated that the self-compartmentalizing HslV peptidase can degrade certain unfolded proteins, such as fully unfolded LA and lysozyme, in the absence of HslU and ATP. Furthermore, HslV alone degraded an LA fragment sandwiched by two thioredoxin molecules, indicating that the peptidase can cleave internal peptide bonds. HslVU protease is a bacterial prototype of the eukaryotic 26S proteasome consisting of the 20S proteasome and the 19S regulatory complex (De Mot *et al*., 1999; Seemuller *et al*., 1995). Crystallographic and biochemical analyses have demonstrated that the 20S proteasome is a gated protease and that opening of the gate is

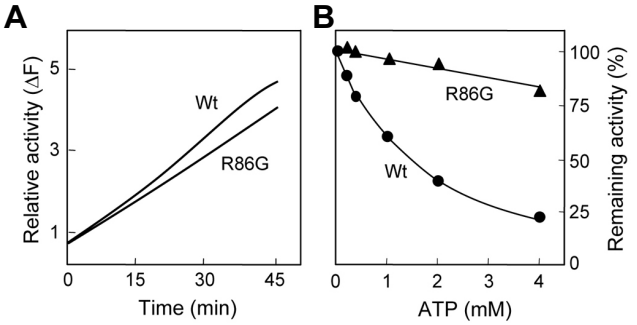

Fig. 5. Effect of the R86G mutation in HslV on peptide hydrolysis. **A.** Wild-type HslV (Wt) or its mutant form (R86G) (20 nM), in which Arg86 was replaced by Gly, was incubated at 37°C with 0.1 mM Z-GGL-AMC and 20 μM C10-peptide. **B.** The mixtures were also incubated at 37°C for 30 min in the presence of increasing concentrations of ATP.

achieved by binding of the 19S regulatory complex or PA28 (Groll *et al*., 2000; Whitby *et al*., 2000). In the latent state of the 20S proteasome, substrate entry into the inner proteolytic chamber is blocked by the gate formed by the N-terminal tails of certain α-subunits that reside in the outer ring. However, in the absence of the 19S regulatory complex the 20S proteasome can degrade u-LA, but not native LA or partially unfolded intermediates (Wenzel and Baumeister, 1995). It has also been demonstrated that the 20S proteasome efficiently degrades the cyclin-dependent kinase inhibitor of p21 and α-synuclein, both of which are known to be "natively disordered" (Liu *et al*., 2003). Furthermore, the 20S proteasome degraded p21 and α-synuclein, each of which were sandwiched by two molecules of non-degradable GFP domains, and so lacked both their N- and C-termini. Based on these findings, it was suggested that the latent 20S proteasome can degrade some natively disordered proteins at internal peptide bonds and that these substrates themselves promote gating of the proteasome (Liu *et al*., 2003). Similarly, certain unfolded proteins, like u-LA and u-lysozyme, may trigger opening of the apical pore of the HslV dodecamer to access the proteolytic chamber.

Noteworthy was the finding that ATP inhibited the degradation of unfolded proteins by HslV whether or not HslU was present. This is in marked contrast to our previous findings that other unfolded proteins, like casein and insulin B-chain, were very poorly degraded by HslV alone, but that their degradation was dramatically stimulated by the presence of HslU and ATP (Rohrwild *et al*., 1996; Seol *et al*., 1997; Yoo *et al*., 1996). A possible explanation for these discrepant results could be a difference in the interaction of two groups of unfolded proteins with HslV and HslU. u-LA and u-lysozyme bind to HslV, but may bind weakly if at all to HslU. On the other hand, casein or the insulin B-chain may interact more strongly with HslU than with HslV, and therefore need to be passed through HslU to reach the inner chamber of HslV peptidase where they are rapidly degraded. Taken together our findings indicate that unfolded protein substrates can be degraded by either uncomplexed HslV or the HslVU complex, respectively, depending on whether they interact preferential with HslV or HslU.

In growing cells, the ATP level is kept constant at millimolar levels. Therefore, uncomplexed HslV is likely to remain in the inactivate state. In this respect, ATP may play an important role in preventing unnecessary breakdown of cellular proteins, such as naturally unstructured proteins, by HslV. However, it has been reported that the ATP pool in *E. coli* changes nearly 10-fold depending on growth rate (Gaal *et al*., 1997). In particular, it is likely that the levels of ATP and other nucleotide triphosphates decrease significantly during starvation of an energy source. In addition, it is well documented that carbon starvation and reduced energy production lead to marked increases in rates of proteolysis to replenish energy sources (Goldberg and St. John, 1976; St. John and Goldberg, 1978). Thus, it is possible that HslV plays a role in degradation of unfolded proteins and thus in replenishment of free amino acids as energy sources under conditions in which the cellular level of ATP is low, such as during carbon starvation.

Acknowledgments We thank Dr. Su-Il Do for providing the cDNA of bovine lactalbumin. This work was supported by grants from the Korea Science and Engineering Foundation (M10533-010001-06N3301-00110 to J.H.S; M10533010001-06N3301-00100 to C.H.C). JWL and EP were recipients of BK21 fellowships supported by the Korean Ministry of Education.

References

Acharya, K. R., Ren, J. S., Stuart, D. I., Phillips, D. C., and Fenna, R. E. (1991) Crystal structure of human alpha-lactalbumin at 1.7 Å resolution. *J. Mol. Biol.* **221**, 571–581.

Bochtler, M., Ditzel, L., Groll, M., and Huber, R. (1997) Crystal structure of heat shock locus V (HslV) from *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **94**, 6070–6074.

Bochtler, M., Hartmann, C., Song, H. K., Bourenkov, G. P., Bartunik, H. D., *et al.* (2000) The structures of HslU and the ATP-dependent protease HslU-HslV. *Nature* **403**, 800–805.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.

Chuang, S. E., Burland, V., Plunkett, G., 3rd, Daniels, D. L., and Blattner, F. R. (1993) Sequence analysis of four new heat-shock genes constituting the *hslTS/ibpAB* and *hslVU* operons in *Escherichia coli*. *Gene* **134**, 1–6.

Chung, C. H. (1993) Proteases in *Escherichia coli*. *Science* **262**, 372–374.

Chung, C. H., Yoo, S. J., Seol, J. H., and Kang, M. S. (1997)

Characterization of energy-dependent proteases in bacteria. *Biochem. Biophys. Res. Commun.* **241**, 613–616.

De Mot, R., Nagy, I., Walz, J., and Baumeister, W. (1999) Proteasomes and other self-compartmentalizing proteases in prokaryotes. *Trends Microbiol.* **7**, 88–92.

Ewbank, J. J. and Creighton, T. E. (1993) Pathway of disulfide-coupled unfolding and refolding of bovine alpha-lactalbumin. *Biochemistry*, **32**, 3677–3693.

Gaal, T., Bartlett, M. S., Ross, W., Turnbough, C. L. Jr., and Gourse, R. L. (1997) Transcription regulation by initiating NTP concentration: rRNA synthesis in bacteria. *Science* **278**, 2092–2097.

Goldberg, A. L. (1992) The mechanism and functions of ATP-dependent proteases in bacterial and animal cells. *Eur. J. Biochem.* **203**, 9–23.

Goldber, A. L. and St. John, A. C. (1976) Intracellular protein degradation in mammalian and bacterial cells: part 2. *Annu. Rev. Biochem.* **45**, 747–803.

Gottesman, S. (1996) Proteases and their targets in *Escherichia coli*. *Annu. Rev. Genet.* **30**, 465–506.

Gottesman, S. (2003) Proteolysis in bacterial regulatory circuits. *Annu. Rev. Cell Dev. Biol.* **19**, 565–587.

Gottesman, S. and Maurizi, M. R. (1992) Regulation by proteolysis: energy-dependent proteases and their targets. *Microbiol. Rev.* **56**, 592–621.

Groll, M., Bajorek, M., Kohler, A., Moroder, L., Rubin, D. M., *et al.* (2000) A gated channel into the proteasome core particle. *Nat. Struct. Biol.* **7**, 1062–1067.

Hiraoka, Y., Segawa, T., Kuwajima, K., Sugai, S., and Murai, N. (1980) Alpha-Lactalbumin: a calcium metalloprotein. *Biochem. Biophys. Res. Commun.* **95**, 1098–10104.

Holt, C. and Sawyer, L. (1988) Primary and predicted secondary structures of the caseins in relation to their biological functions. *Protein Eng.* **2**, 251–259.

Liu, C. W., Corboy, M. J., DeMartino, G. N., and Thomas, P. J. (2003) Endoproteolytic activity of the proteasome. *Science* **299**, 408–411.

Maurizi, M. R. (1992) Proteases and protein degradation in *Escherichia coli*. *Experientia* **48**, 178–201.

Park, E., Rho, Y. M., Koh, O., Ahn, S. W., Seong, I. S., *et al.* (2005) Role of the GYVG pore motif of HslU ATPase in protein unfolding and translocation for degradation by HslV peptidase *J. Biol. Chem*. **280**, 22892–22898.

Park, S. C., Jia, B., Yang, J. K., Van, D. L., Shao, Y. G., *et al.* (2006) Oligomeric structure of the ATP-dependent protease La (Lon) of *Escherichia coli*. *Mol. Cells* **21**, 129–134.

Rohrwild, M., Coux, O., Huang, H. C., Moerschell, R. P., Yoo, S. J., *et al.* (1996) HslV-HslU: A novel ATP-dependent protease complex in *Escherichia coli* related to the eukaryotic proteasome. *Proc. Natl. Acad. Sci. USA* **93**, 5808–5813.

Schagger, H. and von Jagow, G. (1987) Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* **166**, 368–379.

Seemuller, E., Lupas, A., Stock, D., Lowe, J., Huber, R., *et al.* (1995) Proteasome from *Thermoplasma acidophilum*: a threonine protease. *Science* **268**, 579–582.

Seol, J. H., Yoo, S. J., Shin, D. H., Shim, Y. K., Kang, M. S., *et al.* (1997) The heat-shock protein HslVU from *Escherichia coli* is a protein-activated ATPase as well as an ATP-de-pendent proteinase. *Eur. J. Biochem.* **247**, 1143–1150.

Seong, I. S., Oh, J. Y., Yoo, S. J., Seol, J. H., and Chung, C. H. (1999) ATP-dependent degradation of SulA, a cell division inhibitor, by the HslVU protease in *Escherichia coli*. *FEBS Lett.* **456**, 211–214.

Seong, I. S., Oh, J. Y., Lee, J. W., Tanaka, K., and Chung, C. H. (2000) The HslU ATPase acts as a molecular chaperone in prevention of aggregation of SulA, an inhibitor of cell division in *Escherichia coli*. *FEBS Lett.* **477**, 224–229.

Seong, I. S., Kang, M. S., Choi, M. K., Lee, J. W., Koh, O. J., *et al.* (2002) The C-terminal tails of HslU ATPase act as a molecular switch for activation of HslV peptidase. *J. Biol. Chem.* **277**, 25976–25982.

Sousa, M. C., Trame, C. B., Tsuruta, H., Wilbanks, S. M., Reddy, V. S., *et al.* (2000) Crystal and solution structures of an HslUV protease-chaperone complex. *Cell* **103**, 633–643.

St. John, A. C. and Goldberg, A. L. (1978) Effects of reduced energy production on protein degradation, guanosine tetraphosphate, and RNA synthesis in *Escherichia coli. J. Biol. Chem.* **253**, 2705–2711.

Syme, C. D., Blanch, E. W., Holt, C., Jakes, R., Goedert, M., *et al.* (2002) A raman optical activity study of rheomorphism in caseins, synucleins and tau. New insight into the structure and behavior of natively unfolded proteins. *Eur. J. Biochem.* **269**, 148–156.

Wang, J., Hartling, J. A., and Flanagan, J. M. (1998) Crystal structure determination of *Escherichia coli* ClpP starting from an EM-derived mask. *J. Struct. Biol*. **124**, 151–163.

Wang, J., Song, J. J., Franklin, M. C., Kamtekar, S., Im, Y. J., *et al.* (2001a) Crystal structures of the HslVU peptidase-ATPase complex reveal an ATP-dependent proteolysis mechanism. *Structure* **9**, 177–184.

Wang, J., Song, J. J., Seong, I. S., Franklin, M. C., Kamtekar, S., *et al.* (2001b) Nucleotide-dependent conformational changes in a protease-associated ATPase HsIU. *Structure* **9**, 1107–1116.

Wenzel, T. and Baumeister, W. (1995) Conformational constraints in protein degradation by the 20S proteasome. *Nat. Struct. Biol.* **2**, 199–204.

Whitby, F. G., Masters, E. I., Kramer, L., Knowlton, J. R., Yao, Y., *et al.* (2000) Structural basis for the activation of 20S proteasomes by 11S regulators. *Nature* **408**, 115–120.

Yoo, S. J., Seol, J. H., Shin, D. H., Rohrwild, M., Kang, M. S., *et al.* (1996) Purification and characterization of the heat shock proteins HslV and HslU that form a new ATP-de-pendent protease in *Escherichia coli*. *J. Biol. Chem.* **271**, 14035–14040.

Yoo, S. J., Shim, Y. K., Seong, I. S., Seol, J. H., Kang, M. S., *et al.* (1997a) Mutagenesis of two N-terminal Thr and five Ser residues in HslV, the proteolytic component of the ATP-dependent HslVU protease. *FEBS Lett.* **412**, 57–60

Yoo, S. J., Seol, J. H., Seong, I. S., Kang, M. S., and Chung, C. H. (1997b) ATP binding, but not its hydrolysis, is required for assembly and proteolytic activity of the HslVU protease in *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **238**, 581–585.