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Poly-L-lysine Activates both Peptide and ATP Hydrolysis by the ATP-Dependent HslVU Protease in *Escherichia coli*

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HslVU in *E. coli* is a new type of ATP-dependent protease composed of two heat shock proteins, the HslU ATPase and the HslV peptidase related to certain β-type subunits of the 20S proteasome. Here we show that the ATP-dependent hydrolysis of *N*-carbobenzoxy-Gly-Gly-Leu-7-amido-4-methylcoumarin by the HslVU protease can be markedly stimulated by poly-L-lysine, that is known to activate the casein-degrading activity of the 20S proteasome. However, poly-L-lysine showed little or no effect on the peptidase activity of HslV itself. Instead, it stimulated the hydrolysis of ATP by HslU several-fold. Histone that could stimulate the ATPase activity of HslU also increased the rate of the ATP-dependent peptide hydrolysis by HslV, although to a much lesser extent than by poly-L-lysine. Thus, the poly-L-lysine-mediated increase in the ATPase activity of HslU appears to be responsible for the dramatic activation of the ATP-dependent peptide hydrolysis by HslV. These results suggest that, in the reconstituted HslVU complex, the peptide hydrolysis by HslV occurs in a tightly coupled process with the cleavage of ATP by HslU.

E. coli contains a number of ATP-dependent proteases, including proteases La (Lon) and Ti (ClpAP) (1-3). In addition, we have recently described a new type of ATP-dependent protease, the product of the *hslVU* operon, which encodes two heat-shock proteins, HslV and HslU (4-6). While HslU consisting of 6-8 subunit of 50 kDa provides an ATPase activity, HslV that has 12-14 subunits of 19 kDa harbors the peptidase activity, such as against *N*-carbobenzoxy (Cbz)-Gly-Gly-Leu-7-amido-4-methylcoumarin (AMC). In the reconstituted enzyme, HslU markedly stimulates the peptide hydrolysis by HslV (up to 50-fold), while HslV increases the rate of ATP hydrolysis by HslU several-fold (6).

The primary sequence of HslV is similar to that of certain β-type subunits of the 20S proteasome from eukaryotes, certain eubacteria, and the archaebacterium, *Thermoplasma acidophilum* (7). In these enzymes, the *N*-terminal threonine residues of the mature β-type subunits serve as the catalytic site for proteolysis (7, 8). Similarly, HslV has two such conserved *N*-terminal threonine residues (7). Furthermore, its peptidase activity is sensitive to peptide aldehyde inhibitors of the 20S proteasome, such as Cbz-Ile-Glu(*O*-*t*-butyl)-Ala-leucinal or Cbz-Leu-Leu-norvalinal. In addition, it is partially inhibited by lactacystin, a *Streptomyces* metabolite, which covalently modifies the *N*-terminal threonine residue on certain β-type subunits of the 20S proteasome (8). Therefore, it has been suggested that the HslVU protease may function through a similar proteolytic mechanism as the 20S proteasomes, although HslVU does not contain α-type proteasome subunits (6, 7).

An unusual property of the 20S proteasome is that its multicatalytic peptidase activities can be markedly stimulated by low concentrations of SDS and fatty acids (9). In addition, the 20S proteasome by itself can degrade certain proteins, such as casein, in the presence of poly-L-lysine, but not in its absence (10). Therefore, it has been suggested that the 20S proteasome

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TABLE 1
Effects of Poly-L-lysine and SDS on the Hydrolysis of Cbz-Gly-Gly-Leu-AMC by HsIV

Addition	Concentration	pmol Hydrolyzed/h	% Relative activity
Poly-L-lysine	None	3.8	100
	1 μg/ml	4.2	111
	10 μg/ml	4.5	118
SDS	0.002%	1.8	47
	0.02%	0.1	3

Note. Peptide hydrolysis was assayed by incubating 1 mM ATP and 0.05 μg of HsIV in the presence and absence of poly-L-lysine or SDS for 1 h at 37°C as described under Materials and Methods.

is a latent protease (9, 10). Since the catalytic mechanism for the 20S proteasome appears similar to that for HslV in addition to the similarity in their structure (5, 6), the present studies were performed to determine whether poly-L-lysine or SDS may influence on the ability of the HslVU protease to hydrolyze peptides and ATP.

MATERIALS AND METHODS

Materials. The purified HslV and HslU were prepared as described previously (6). Peptide substrates were obtained from BACHEM Feinchemikalien AG (Switzerland). All other reagents, including poly-L-lysine, were purchased from Sigma.

Assays. Peptide hydrolysis was assayed as described previously (5, 6) using Cbz-Gly-Gly-Leu-AMC as the substrate in the presence and absence of poly-L-lysine. Reaction mixtures (0.1 ml) contained the peptide (0.1 mM) and appropriate amounts of the purified HslV and HslU or HslV alone in 0.1 M Tris-HCl buffer (pH 8) containing 10 mM $MgCl_2$, 1 mM ATP, 1 mM dithiothreitol and 1 mM EDTA. Incubations were performed for 10-60 min at 37 °C and stopped by adding 0.1 ml of 1% (w/v) SDS and 0.8 ml of 0.1 M sodium borate (pH 9.1). The release of AMC was then measured using a fluorometer. To assay ATP hydrolysis, similar reaction mixtures were incubated as above in the presence and absence of poly-L-lysine. After incubation, 0.2 ml of 1% SDS was added to the samples, and the phosphate produced was determined as described (11). Proteins were assayed by their absorbance at 280 nm or by the method of Bradford (12) using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

HslV is a weak peptidase that can be markedly activated by HslU and ATP (6). Therefore, we first examined whether poly-L-lysine or SDS may influence on the peptidase activity of HslV alone. As shown in Table 1, poly-L-lysine showed little or no effect on the hydrolysis of Cbz-Gly-Gly-Leu-AMC by HslV. On the other hand, the peptide cleavage by HslV was strongly inhibited by SDS, unlike the 20S proteasome, whose multicatalytic peptidase activity is dramatically stimulated by SDS at the concentrations tested (data not shown; 9, 10).

We then examined the effect of poly-L-lysine on the peptide hydrolysis by HslV in the presence of HslU and ATP. As shown in Fig. 1A, poly-L-lysine dramatically stimulated the ATP-dependent peptide hydrolysis by the HslVU protease (*i.e.*, the mixture of HslU and HslV). Furthermore, poly-L-lysine stimulated the peptide-degrading activity of the enzyme in a concentration-dependent fashion until to reach a maximal effect (Fig. 1B). On the other hand, SDS inhibited the peptide hydrolysis whether or not HslU and/or ATP is present (data not shown). Thus, the apparent mechanism for the activation of the 20S proteasome by the reagents appears distinct from that for the HslVU protease.

Since poly-L-lysine does not appear to directly interact with HslV itself, we suspected whether the poly-cationic agent may influence on the ATPase activity of HslU. To test this possibility, we first examined the effect of poly-L-lysine on ATP hydrolysis by HslU alone, since HslU is an inherent ATPase that can be stimulated by HslV (6). As shown in Fig. 2A

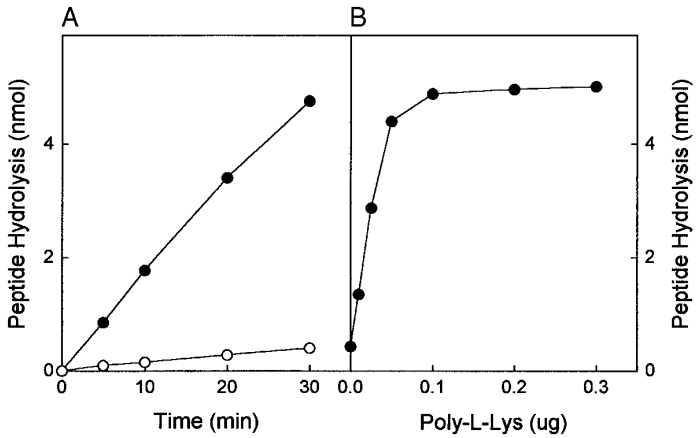

FIG. 1. Effect of poly-L-lysine on ATP-dependent hydrolysis of Cbz-Gly-Gly-Leu-AMC by the HslVU protease. (A) Peptide hydrolysis was assayed by incubating 1 mM ATP, 0.2 μg of HslU, and 0.05 μg of HslV in the absence (○) and presence (●) of 1 μg of poly-L-lysine for various periods at 37 °C. (B) Assays were also carried out by incubating the same amounts of the proteins in the presence of increasing amounts of poly-L-lysine for 30 min at 37 °C.

(*triangles*), poly-L-lysine increased the rate of ATP hydrolysis by HslU about 5-fold. In the presence of HslV, the basal ATPase activity of HslU was stimulated by HslV by about 3-fold, and this increased activity was further stimulated by about 5-fold upon incubation with poly-L-lysine (Fig. 2A, *circles*). Furthermore, poly-L-lysine stimulated the ATP hydrolysis by the HslVU protease in a concentration-dependent fashion, similar to its effect on the ATP-dependent peptide-cleaving activity of the enzyme (Fig. 2B). However, the absolute degree of the stimulation by poly-L-lysine was approximately the same (*i.e.*, about 5-fold) whether or not HslV was present, indicating that poly-L-lysine interacts with HslU but not with HslV. In addition, poly-L-lysine did not change the affinity of HslU to ATP (*i.e.*, the K_m value) whether

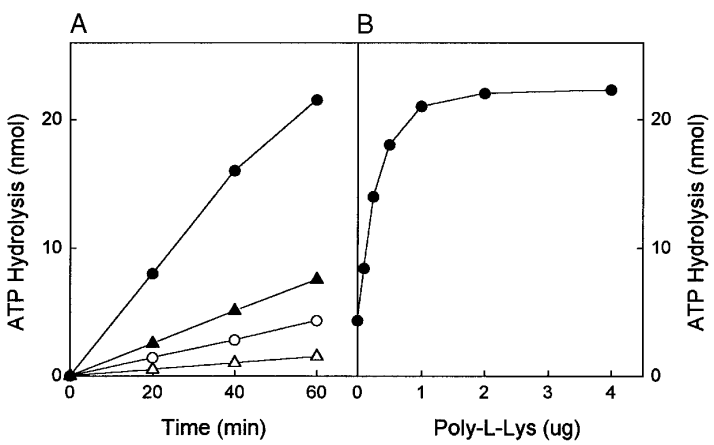

FIG. 2. Effect of poly-L-lysine on ATP hydrolysis by HslU in the presence and absence of HslV. (A) ATP hydrolysis was assayed by incubating 1 mM ATP and 0.8 μg of HslU alone (△), HslU plus 1 μg of poly-L-lysine (▲), HslU plus 0.2 μg of HslV (○), or all together (●) for various periods at 37 °C. (B) Assays were also performed by incubating 1 mM ATP, 0.8 μg of HslU, and 0.2 μg of HslV in the presence of increasing amounts of poly-L-lysine for 1 h at 37 °C.

TABLE 2
Effects of Various Poly-cationic Agents on the Hydrolysis of Cbz-Gly-Gly-Leu-AMC and ATP by the HsIVU Protease

	Hydrolysis (nmol/h) of	
Addition	Cbz-Gly-Gly-Leu-AMC	ATP
None	0.7	4.9
Poly-L-Lys	11.0	23.6
Poly-L-Arg	0.8	5.2
Histone	1.9	9.4
Spermine	0.7	5.0
Spermidine	0.7	4.9
Putrescine	0.6	4.5

Note. Peptide and ATP hydrolysis were assayed as described in Fig. 2 but in the absence and presence of 1 μg of various poly-cationic agents, except that histone was added with 10 μg. Incubations were performed for 1 h at 37°C.

or not HslV was present (data not shown). Thus, it appears likely that the increase in the ATPase activity of HslU by poly-L-lysine is responsible for the dramatic activation of the ATP-dependent peptide hydrolysis by HslV. However, the stoichiometric relationship for ATP hydrolysis per peptide bond cleavage is unclear at present, because approximately 5-fold increase in the ATPase activity of HslU by poly-L-lysine results in up to 20-fold stimulation of the peptide cleavage by HslV.

In order to determine whether other poly-cationic agents may also stimulate the ATPase activity of HslU and hence the ATP-dependent peptide hydrolysis by HslV, both ATP and peptide hydrolysis were assayed by incubating the mixture of HslU and HslV with and without the reagents. As shown in Table 2, a maximal stimulatory effect was observed with poly-L-lysine. Histone also stimulated the activities, although to an extent that is much less than that seen with poly-L-lysine. In addition, approximately 10-fold higher amounts of histone than poly-L-lysine were required to observe the stimulatory effect. On the other hand, poly-L-arginine showed little or no effect on either of the ATPase or peptidase activity of the HslVU protease. None of the other small cationic molecules (*e.g.*, spermine, spermidine or putrescine) showed any effect. These results clearly indicate that the stimulatory effect of poly-L-lysine is not due to simple, nonspecific charge effect, although the mechanism by which poly-L-lysine stimulates the process requiring ATP hydrolysis remains totally unknown.

We have previously shown that only the nucleoside triphosphates that were hydrolyzed by HslU, such as ATP, dATP and CTP, can activate the hydrolysis of Cbz-Gly-Gly-Leu-AMC by HslV and that the degree of the activation is proportional to the extent of the hydrolysis of the nucleotides (5, 6). Therefore, we have suggested that the hydrolysis of ATP and peptide occurs in a linked process (5, 6). The present findings that the poly-L-lysine-mediated increase in ATP hydrolysis results in stimulation of the ATP-dependent peptide cleavage further support our suggestion that peptide hydrolysis is tightly coupled with ATP cleavage.

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