生物化学与生物物理进展 *Progress in Biochemistry and Biophysics*, 2006, 33(2): 183~189
www.pibb.ac.cn

Peptide Induced Conformational Changes of *E. coli* DegP (HtrA) Protease*

ZHANG Xue-Feng[1)], ZHENG Yi-Xin[2,3)], CHANG Zeng-Yi[1,2,3)]**

([1)]*Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing* 100084, China;
[2)]*National Key Laboratory of Protein Engineering and Plant Genetic Engineering, Peking University, Beijing* 100871, China;
[3)]*College of Life Science, Peking University, Beijing* 100871, China)

Abstract The DegP protein, functioning as both chaperone and protease, plays a critical role in degrading and removing denatured or damaged proteins in the cellular envelope during heat shock and other stresses. So far, several proteins have been identified as its natural targets. A carboxyle-terminal peptide derived from the PapG pilus, one of the *in vivo* substrates for DegP, has been shown to activate the protease. Nevertheless, neither the details nor the physiological implications of such activation have been studied. The evidence that DegP undergoes conformational changes upon binding the peptide derived from C-terminal sequence of pilus subunit PapG has been presented. It demonstrated that upon binding this peptide, detectable changes can be observed for both secondary and tertiary structures of DegP, as examined by CD spectroscopy. Gel filtration and dynamic light scattering analysis also revealed that the size of DegP becomes smaller to a minor extent. Moreover, both the hydrophobic surfaces and catalytic sites of DegP were found to expose slightly in the presence of the peptide. Upon peptide binding, a less flexible and more rigid conformation of DegP was obtained as analyzed by fluorescence anisotropy. The physiological implications of these observations for DegP are discussed.

Key words DegP, conformational change, peptide, mechanism

HtrA is a highly conserved family of oligomeric serine proteases, members of which are found in most organisms, including human. The structural feature of HtrA family is the combination of a trypsin-like catalytic domain with at least one C-terminal PDZ domain [1]. PDZ domains are composed typically of 80～100 amino acids and have been reported in many proteins involved in a range of protein：protein interactions. The abbreviation PDZ derived from three eukaryotic proteins, Post-synaptic density protein, Disc large, and Zonula occludens[2].

E.coli HtrA, also known as DegP, is indispensable for cell survival at elevated temperatures[3]. DegP is a peripheral membrane protein localized on the periplasmic side of inner membrane[4]. It functions as a protease at high temperatures and as a chaperone at low temperatures[5]. The physiological role of DegP is to degrade and remove abnormally folded or damaged proteins in the cellular envelope during heat shock and other stresses [6～8]. DegP is synthesized as a 51 ku preprotein from which a signal peptide of 26 amino acids is removed by signal peptidase [3,9]. Mature DegP is a 48 ku protein and can undergo partial autocleavage [10]. According to the resolved crystal structure [11], DegP is a hexamer formed by staggered association of two trimeric rings. Top and bottom of the molecular cage are constructed by the six protease domains, whereas the mobile side-walls are formed by twelve PDZ domains. The proteolytic sites are located on the inner wall of the central cavity.

To date, four proteins have been described as DegP substrates *in vivo*: colicin A lysis protein[12], pilus subunits [13], MalS protein of *E.coli* [5], and highmolecular-weight adherence proteins HMW1, and HMW2 from *Haemophilus influnzae*[14]. *In vitro*, DegP acts as general protease, it can digest many unrelated proteins such as casein, PhoA, MBP and recombinant proteins. All these substrates have in common that they are at least partially unfolded[1].

*This work was supported by grants from The National Basic Research Program of China (G1999075607) and The National Natural Science Foundation of China (30270289).
**Corresponding author . Tel: 86-10-62758822, Fax: 86-10-62751526, E-mail: changzy@pku.edu.cn
Received: September 22, 2005 Accepted: November 1, 2005

Recently, Jones *et al.*[15] demonstrated that a 10-mer nonecleaveable peptide from the conserved carboxyl-terminal sequence of pilus subunit PapG, activates the DegP protease. However, neither the details nor the physiological implications of such an activation of DegP have been studied. Here we present evidence that DegP undergoes conformational changes upon binding the peptide derived from C-terminal sequence of pilus subunit PapG. This conformational change may have important implications for the mechanism of DegP activation and the understanding of the physiological functions of this protease.

1 Materials and methods

1.1 Materials

Bovine β - casein, 1 - anilinonaphthalene - 8 - sulfonate (ANS) were purchased from Sigma (USA). Lucifer Yellow iodoacetamide (LYI) was obtained from Molecular Probes, Eugene, OR. The 5 ml desalting column was purchased from Amersham Pharmacia Biotech (USA). The carboxyl-terminal peptide of PapG (PapG-C10) was synthesized and purified by Shanghai Sangon Ltd. The sequence is as follows: KSMCMKLSFS. All other chemical reagents were of analytical purity.

1.2 Plasmid construction and protein purification

To generate the DegP protease with a C-terminal polyhistidine tag, a PCR reaction was conducted using Ptrc99a containing the wild-type DegP gene (a generous gift from Dr. Betton, France) as a template. The PCR product was digested with *Nco* Ⅰ and *Xho* Ⅰ restriction enzymes and then was ligated into pET-28a predigested with the above two enzymes. To construct mutant DegPS210A and DegPS210W in which active site Ser210 was changed to alanie and tryptophan, respectively, overlap PCR was performed. The PCR products were ligated into pET-28a. All the resultant plasmids were verified by DNA sequencing.

To express and purify wild-type and mutant DegP, cells were grown at 37℃ in Luria-Bertani (LB) medium (plus 50 mg/L kanamycine) and then induced (A_{600} of 0.8～1) with 0.2 mmol/L isopropyl-1-thio-β-D-galactopyranoside (IPTG) for 4 h at 37℃. After centrifugation at 5 000 r/min for 15 min, the cell pellet was resuspended in a buffer containing 50 mmol/L Na_2HPO_4-NaH_2PO_4, pH 7.6, 50 mmol/L NaCl, 20 mmol/L imidazole. The cells were disrupted by sonication, and then the cell lysate was centrifuged for 50 min at 15 000 r/min. The supernatant was loaded onto a 4 ml Ni-NTA agarose column. The column was then washed with 100 ml buffer containing 50 mmol/L Na_2HPO_4-NaH_2PO_4, pH 7.6, 0.5 mol/L NaCl, 20 mmol/L imidazole. This was followed by the same volume of buffer containing 50 mmol/L Na_2HPO_4-NaH_2PO_4, pH 7.6, 1 mol/L NaCl, 20 mmol/L imidazole. DegP and its mutants were eluted with buffer containing 50 mmol/L Na_2HPO_4-NaH_2PO_4, pH 7.6, 50 mmol/L NaCl, 250 mmol/L imidazole. Purity (> 95%) of the fractions was controlled by SDS-PAGE stained with Coomassie brilliant blue.

1.3 Circular dichroism measurements

Far and near-UV circular dichroism (CD) spectra of DegPS210A were measured in the absence or in the presence of PapG-C10 peptide on a Jasco J-715 (Japan) spectropolarimeter equipped with a constant temperature cell holder. The spectra were recorded using 2-mm path length cells. Protein concentrations of 0.3 g/L and 3 g/L were used for far and near-UV CD spectra measurements, respectively. All the spectra were cumulative averages of 10 repeated scans.

1.4 Size exclusion chromatography

Size exclusion chromatography (SEC) analysis was performed on a ÄKTAFPLC system using pre-packed Superdex 200 10/30 column (all from Amersham-Pharmacia Biotech). For each analysis, a 100 μl sample (of 1 g/L) was loaded and eluted with 50 mmol/L Na_2HPO_4-NaH_2PO_4 (pH 7.6, 50 mmol/L NaCl) at a flow rate of 0.35 ml/min.

1.5 Dynamic light scattering (DLS) experiments

The protein was diluted to 1.0 g/L and centrifuged at 15 000 r/min for 30 min. Samples of 500 μl supernatant were measured in a ALV/CGS-5022F (ALV/Laser Vertriebsgesells chaft m.b.H, Germany) at 25℃. From the measured translational diffusion coefficient D_T, the hydrodynamic radius R_H can be calculated using the Stokes-Einstein equation: $D_T=K_BT/6\pi\eta R_H$, with the Boltzmann constant K_B, the temperature T in Kelvin and η being the viscosity of the solvent.

1.6 ANS and intrinsic fluorescence

For ANS fluorescence spectra, the excitation wavelength was set at 390 nm and the emission was scanned from 400 to 600 nm. The final concentrations for DegPS210A and ANS were 2 and 100 μmol/L, respectively. For intrinsic fluorescence measurements, samples were excited at 280 nm and emission spectra were recorded from 300 to 400 nm. Spectra were recorded at room temperature on a Hitachi P-4500

fluorescence spectrophotometer.

1.7 Labeling of DegPS210A with fluorescence probes

DegPS210A was first reduced by DTT, and then diluted to 1 g/L in 50 mmol/L Na_2HPO_4-NaH_2PO_4, pH 7.6, containing 50 mmol/L NaCl. Solid LYI was added to the solution to final concentration of 1 mmol/L. The reaction was allowed to proceed at room temperature in the dark for 12 h. Covalently bound probe was separated from free, unreacted probe using gel filtration with a Sephadex G-25 column.

1.8 Fluorescence anisotropy

Fluorescence anisotropy was used to study the mobility of DegPS210A in the presence PapG-C10 peptide. LYI-DegPS210A was co-incubated with PapG-C10 peptide for 1 h before being applied for fluorescence anisotropy analysis. The emission spectrum of LYI labeled DegPS210A was recorded at 25℃ on a Hitachi P-4500 fluorescence spectrophotometer between 500 nm and 540 nm (excited at 435 nm), with the anisotropy being calculated by following equation: $R=(I_{VV}-GI_{VH})/(I_{VV}+2GI_{VH})$, $G=I_{HV}/I_{HH}$, where I_{VV} and I_{VH} are fluorescence intensities that were measured with vertically polarized excitation, detecting through vertically and horizontally oriented emission polarizers, respectively. The factor G, which was measured using horizontally polarized excitation, corrects for instrument polarization. Each result was the average of two parallel experiments.

2 Results

2.1 PapG-C10 peptide enhances the protease activity of DegP

Previous work [15] unexpectedly revealed that the C-terminal sequence of the pilus subunit PapG (PapG-C10) is able to specifically activate the protease activity of DegP towards the pilus subunit PapA. Here using β-casein as the substrate, we assayed the protease activity of purified mature DegP in the presence of this peptide. As expected, the peptide also enhanced the protease activity of DegP in a concentration-dependent manner (Figure 1). The peptide itself had no proteolytic effect on β-casein (Figure 1, lanes *6*～*7*). The most obvious increase in the protease activity was observed with the concentration of PapG-C10 at 60 μmol/L, at which the molar ratio of DegP to peptide being 1 ∶20, a condition adopted for the subsequent experiments.

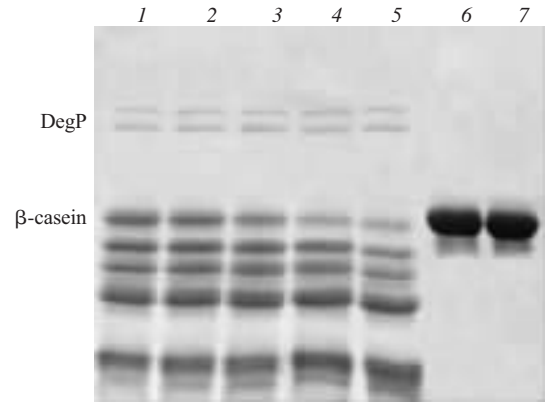

Fig. 1 Activation of DegP protease by PapG-C10 peptide

β-casein (20 μg) was incubated with purified DegP (1 μg) (lanes *1*～*5*) in the presence of indicated concentrations of PapG-C10 peptide. The incubation was carried out at 30℃ for 60min in a reaction volume of 15 μl. The reaction products were analyzed by SDS-PAGE. *1*: 0 μmol/L; *2*: 1.5 μmol/L; *3*: 15 μmol/L; *4*: 30 μmol/L; *5*: 60 μmol/L; *6*: 60 μmol/L; *7*: 0 μmol/L.

2.2 Binding of PapG-C10 peptide affects the structure of DegP

To get more insight into the mechanism of DegP activation by PapG-C10 peptide, structural changes of

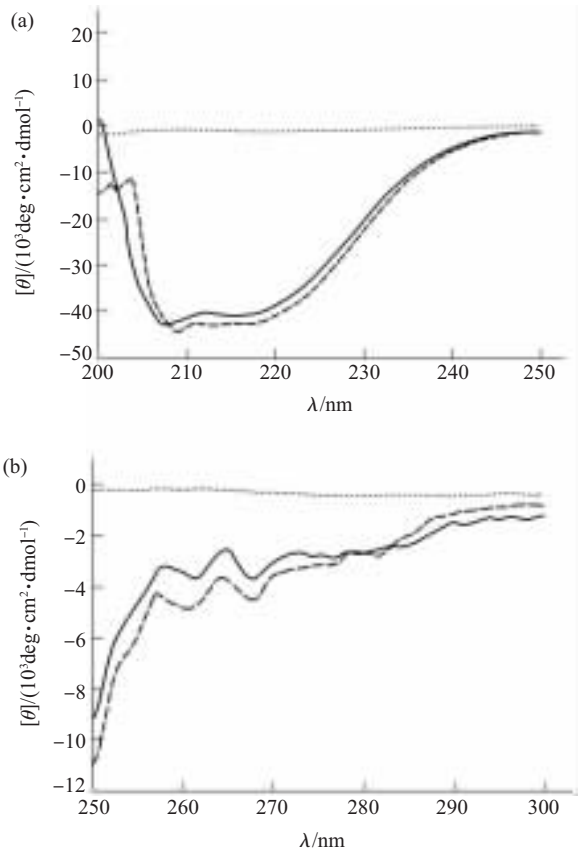

Fig. 2 far-UV CD spectra (a) and near-UV CD spectra (b) of DegPS210A in the presence or absence of PapG-C10 peptide

——: DegPS210A; -------: +peptide; ·····: Peptide alone.

DegP upon binding the peptide were examined via circular dichroism (CD) and size-exclusion chromatography. Given that the native DegP protein undergoes slow self-cleavage [10], a mutant protein DegPS210A (in which the active site serine was replaced by an alanine) that does not change the overall structure of DegP [10] was applied for these analyses. The recorded far-UV CD spectra (Figure 2a) demonstrated that there were small but significant difference between the secondary structures of free DegPS210A and DegPS210A with PapG-C10 peptide bound. The far-UV CD spectra of DegPS210A showed a 2～3 nm red shift of the negative maximum, and an increase in the amount of α-helical content (from 17.8% to 22.1%) after binding the PapG-C10 peptide. The near-UV CD spectra (Figure 2b) of DegP also changed significantly in the presence of the peptide, having an increase in the negative ellipticity.

Size-exclusion chromatography analysis revealed a minor but repeatedly detectable increase in the elution volume for DegP-S210A in the presence of the PapG-C10 peptide (Figure 3), indicating a small decrease in the size of the protein. Consistent with this observation is the results of dynamic light scattering (DLS) analysis, which indicate a decrease of the effective hydrated radius for DegPS210A in the presence of the PapG-C10 peptide (Table 1).

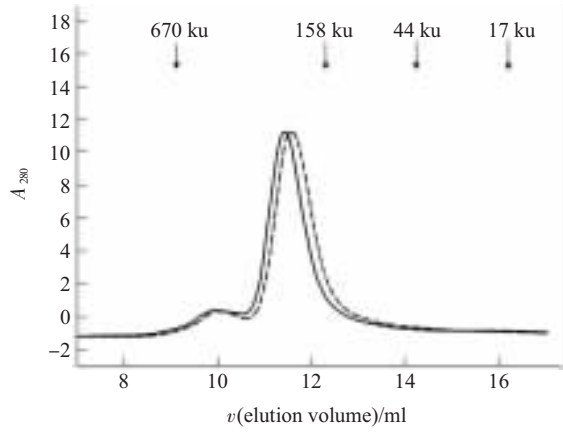

Fig. 3 Peak profiles in gel filtration chromatography of DegPS210A in the presence or absence of PapG-C10 peptide

The arrows indicate the positions of molecular mass standard (thyroglobulin, 670 ku; bovine gamma globulin, 158 ku; chicken ovalbumin, 44 ku; equine myoglobin, 17ku). ——: DegPS210A; -------: +peptide.

Table 1 Results of dynamic light scattering measurements on DegPS210A

Samples	λ/nm	D_T/10^{-7}cm^2·s^{-1}	R_H/nm
DegPS210A	632.8	2.22(0.12)	11.04(0.17)
DegPS210A+peptide	632.8	2.57(0.10)	9.55(0.13)

standard deviation was shown between brackets.

2.3 DegP exposes hydrophobic surfaces upon binding the peptide

ANS has been widely used as a fluorescence probe to demonstrate the presence of hydrophobic patches on protein surfaces. The fluorescence intensity usually increases when ANS binds to hydrophobic protein surfaces[16, 17]. It has been reported that increased proteolytic activity of DegP at elevated temperatures correlates with an increased amount of hydrophobic surface [6]. Are the enhanced proteolytic activities of peptide bound DegP accompanied by exposure of hydrophobic surfaces? ANS was thus used to probe the exposure of such hydrophobic surfaces on DegP upon peptide binding. The results, shown in Figure 4, demonstrated that the fluorescence intensity of ANS interacting with DegPS210A in the presence of the PapG-C10 peptide increased, and the maximal emission wavelength of ANS bound to DegPS210A was 5 nm blue- shifted, indicating that peptide binding results in some hydrophobic areas of DegP.

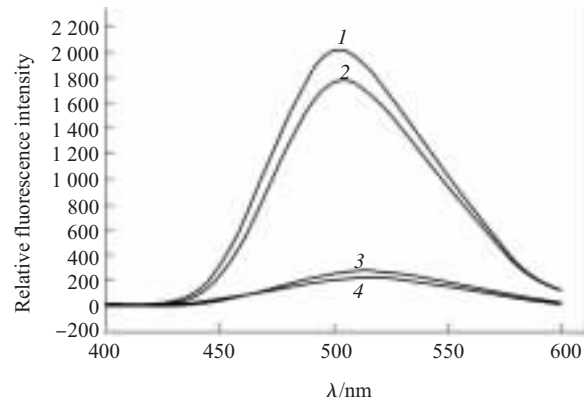

Fig. 4 ANS-binding fluorescence spectra

Curves *1* ～*4* represent the ANS-binding fluorescence spectra for DegPS210A in the presence of PapG-C10 peptide, DegPS210A, PapG-C10 peptide, and ANS alone, respectively.

2.4 The intrinsic fluorescence of DegP changes upon binding the peptide

In order to further characterize the effect of peptide binding on the conformation of DegP, intrinsic fluorescence spectra were recorded. DegP possesses no Trp residues but five Tyr residues, the PapG-C10 peptide has no Trp residues either. To detect whether conformational changes occur near the catalytic site in

DegP, the DegPS210W mutant was constructed, in which the catalytic residue Ser210 was replaced by a Trp. This mutation did not significantly change the whole structure of DegP as proved by size-exclusion chromatography analysis and CD (data not shown). Because of the lack of tryptophan residues in the PapG-C10 peptide, changes in the intrinsic fluorescence for DegPS210W in the presence of the peptide would mainly reflect conformational changes around this introduced Trp residue. Data presented in Figure 5 demonstrated a slight decrease in the intensity of the intrinsic fluorescence for DegPS210W in the presence of the PapG-C10 peptide, with about 3 nm red-shift. These results suggest that the environment of Trp210 in DegPS210W with peptide bound is less hydrophobic than that in DegPS210W alone, indicating the exposure of catalytic sites of DegP to some extent.

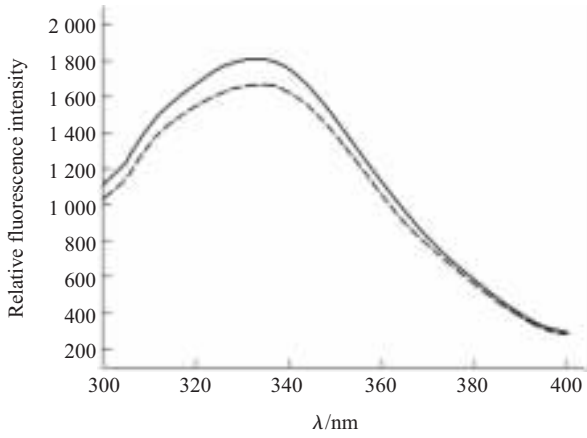

Fig. 5 Intrinsic fluorescence spectra of DegPS210W (50 mg/L) in the presence or absence of PapG-C10 peptide

——: DegPS210W; -------: +peptide.

2.5 The conformation of DegP becomes less flexible in the presence of the PapG-C10 peptide

To further investigate the nature of the structural changes revealed by the above results, fluorescence anisotropy, a widely used technique for measuring the size, shape, and flexibility of macromolecules[18,19] was analyzed for DegP upon binding the peptide. The results demonstrated that the anisotropy values for free DegP and peptide bound DegP were 0.025 and 0.035, respectively. Since the size of DegP appears to become slightly smaller (Figure 3 and Table 1) upon binding the peptide, such increased anisotropy most likely reflect a reduction in the conformational flexibility of DegP, which would lead to a more limited rotation for the covalently linked probe.

3 Discussion

DegP belongs to the superfamily of HtrA serine protease. It contains a central trypsin-like protease domain followed by two C-terminal PDZ domains[5]. The most striking feature of the motif recognized by PDZ domain is that the residue at −3 position is often a conserved residue (threonine, serine, or tyrosine)[20,21]. An alignment of the C-terminus of pilus subunits revealed that the −3 position is often a serine, threonine, or tyrosine[22]. Moreover, studies have revealed that PDZ domains of *E.coli* DegP and Tsp protease participate in specific substrate recognition[23,24]. In light of these, the PapG-C10 peptide with a sequence of KSMCMKLSFS and being derived from the C-terminus of a pilus subunit PapG, has been suggested to bind to the PDZ domains of DegP and thus enhancing the protease activity of the protein[15]. This recognition is specific because other irrelevant peptides failed to exhibit similar effect[15].

In this study, we have shown that the conformations of DegP and peptide bound DegP are different. The CD spectra (Figure 2) reveal a detectable change in both the secondary and tertiary structures of DegP upon binding the PapG-C10 peptide. Since it is possible that the PapG-C10 peptide itself could contribute a small signal to the far-UV or near-UV CD spectra, we cannot absolutely be certain that the observed changes do result from a peptide-induced changes in the secondary and tertiary structures. However, in light of CD spectra of free PapG-C10 peptide and the conformational changes observed by the other methods used in this study, it is most likely that the presence of peptide induces the changes in the secondary and tertiary structures of DegP.

The exposure of hydrophobic surfaces and catalytic sites for DegP protein upon peptide binding (Figure 4, 5) provides strong evidence for understanding the mechanism of DegP activation. The crystal structure of DegP reveals that the proteolytic sites of DegP hexamer are located in a central cavity, and the inner cavity is lined by several hydrophobic patches that act as docking sites for unfolded polypeptides[11]. An increased amount of hydrophobic surface has been reported to correlate with increased proteolytic activity of DegP at elevated temperatures[6]. Thus, peptide induced exposure of hydrophobic

surfaces and catalytic sites for DegP may facilitate substrate binding and subsequent moving to the catalytic sites, leading to an increase in the protease activity. Whether the smaller size of DegP (Figure 3 and Table 1) resulting from peptide binding correlates with the enhanced protease activity is not clear. We propose that the smaller size of DegP molecule may shorten the distance between the substrates binding sites and catalytic sites, which may in turn promote substrate moving to the catalytic sites more easily.

The affect of the protease activity of DegP by PapG-C10 peptide is comparable to the activation process of DegS, another protease present in the periplasm of *E.coli*. DegS is present in a nonfunctional state when the activation peptide is not bound to its PDZ domain[25,26]. Binding of the activation peptide to the PDZ domain induces a conformational change in the flexible L3 loop, which in turn triggers a reorientation of the activation domain yielding a functional active site, thus promoting degradation of the substrates[26]. DegS was also found to become less flexible and more rigid upon binding the activation peptide[26], which is consistent with our observations reported here.

It was recently reported that the PDZ domains of both mouse HtrA1 and Human HtrA2 are recognized by specific peptides, resulting in an increase in proteolytic activity[27,28]. An activation mechanism similar to DegS has been considered for both proteases[27,28]. Since members of HtrA family share homologous protease and PDZ domains, it may be asked whether such a activation mechanism is a general property for this family of protease. Further exploration of this issue may bring us more insights into the action mechanism of HtrA molecules, and may also help us to understand their physiological functions as well.

Our results combined with previous reports imply a possible physiological function of DegP in monitoring biogenesis of bacterial pili. The stability of pilus subunits in the periplasm is dependent on the activity of the PapD chaperone[29,30]. In the absence of chaperone, the subunits are prone to aggregation[29]. DegP may recognize denatured pilus subunits through the exposed C-terminal ends of pilus (similarly as DegS) and trigger a reorientation of the protease domain for DegP. This conformational change will cause DegP to digest misfolded pilus subunits.

Acknowledgments The authors are grateful to the helpful discussions provided by members of Chang's Laboratory.

References

1 Clausen T, Southan C, Ehrmann M. The HtrA family of proteases: implications for protein composition and cell fate. Mol Cell, 2002, **10** (3): 443～455

2 Faccio L, Fusco C, Chen A, *et al*. Characterization of a novel human serine protease that has extensive homology to bacterial heat shock endoprotease HtrA and is regulated by Kidney Ischemia. J Biol Chem, 2000, **275** (4): 2581～2588

3 Lipinska B, Fayet O, Baird L, *et al*. Identification, characterization, and mapping of the *Escherichia coli htrA* gene, whose product is essential for bacterial growth only at elevated temperatures. J Bacteriol, 1989, **171** (3): 1574～1584

4 Skorko-Glonek J, Lipinska B, Krzewski K, *et al*. HtrA heat shock protease interacts with phospholipid membranes and undergoes conformational changes. J Biol Chem, 1997, **272** (14): 8974～8982

5 Spiess C, Beil A, Ehrman M. A temperature dependent switch from chaperone to protease in a widely conserved heat shock protein. Cell, 1999, **97** (3): 339～347

6 Kim K, Park S, Kang S, *et al*. Selective degradation of unfolded proteins by the self-compartmentalizing HtrA protease, a periplasmic heat shock protein in *Escherichia coli*. J Mol Biol, 1999, **294** (5): 1363～1374

7 Kolmar H, Waller P, Sauer R. The degP and degQ periplasmic endoprotease of *Escherichia coli*: specificity for cleavage sites and substrate conformation. J Bacteriol, 1996, **178** (20): 5925～5929

8 Skorko-Glonek J, Zurawa D, Kuczwara E, *et al*. *Escherichia coli* heat shock HtrA protease participates in the defense against oxidative stress. Mol Genet Genomics, 1999, **262** (2): 342～350

9 lipinska B, Sharma S, Georgopoulos C. Sequence analysis and regulation of the *htrA* gene of *Escherichia coli*: a sigma 32-independent mechanism of heat-inducible transcription. Nucleic Acids Res, 1988, **16** (21): 10053～10067

10 Skorko-Glonek J, Wawrzynow A, Krzewski K, *et al*. Site-directed mutagenesis of the HtrA (DegP) serine protease, whose proteolytic activity is indispensable for *Escherichia coli* survival at elevated temperatures. Gene, 1995, **163** (1): 47～52

11 Krojer T, Garrido-Franco M, Huber R, *et al*. Crystal structure of degP (HtrA) reveals a new protease-chaperone machine. Nature, 2002, **416** (6879): 455～459

12 Cavard D, Lazdunski C, Howard S P. The acylated precursor form of the colicin A lysis protein is a natural substrate of the DegP protease. J Bacteriol, 1989, **171** (3): 6316～6322

13 Jones C, Danese P, Pinkner J. The chaperone-assisted membrane release and folding pathway is sensed by two signal transduction systems. EMBO J, 1997, **16** (7): 6394～6406

14 Geme S, Grass S. Secretion of the Haemophilus influnzae HMW1 and HMW2 adhesins involves a periplasmic intermediate and requires the HMWB and HMWC proteins. Mol Microbiol, 1998, **27** (3): 617～630

15 Jones C, Dexter P, Evans A. *Escherichia coli* DegP protease cleaves between paired hydrophobic residues in a natural substrate: the PapA pilin. J Bacteriol, 2002, **184** (20): 5762～5771

16 Mizobata T, Kawata Y. The guanidine-induced conformational changes of the chaperonin GroEL from *Escherichia coli*. Evidence for the existence of an unfolding intermediate state. Biochim Biophys Acta, 1994, **1209** (1): 83～88

17 Das B K, Liang J. Detection and characterization of alpha-crystallin intermediate with maximal chaperone-like activity. Biochem Biophys Res Commun, 1997, **236** (2): 370～374

18 Fu X, Chang Z. Temperature-dependent subunit exchange and chaperone-like activities of Hsp16.3, a small heat shock protein from *Mycobacterium tuberculosis*. Biochem Biophys Res Commun, 2004, **316** (2): 291～299

19 Lakowicz J. Principles of Fluorescence Spectroscopy. 2nd. New York: Kluwer Academic/Plenum Publishers, 1999. 321～345

20 Harrison S. Peptide-surface association: the case of PDZ and PTB domains.Cell, 1996, **86** (3): 341～343

21 Songyang Z, Fanning A, Fu C, *et al*. Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. Science, 1997, **275** (5296): 73～77

22 Soto G E, Hultgren S J. Bacterial adhesions: common themes and variations in architecture and assembly. J Bacteriol, 1999, **181**(4): 1059～1071

23 Beebe K D, Shin J, Peng J, *et al*. Substrate recognition through a PDZ domain in Tail-specific protease. Biochemistry, 2000, **39** (11): 3149～3155

24 Spiers A, Lamb H K, Cocklin S, *et al*. PDZ domains facilitate binding of high temperature requirement protease (HtrA) and tail-specific protease (Tsp) to heterologous substrates through recognition of small stable RNA A (ssrA)-encoded peptide. J Biol Chem, 2002, **277** (42): 39443～39449

25 Walsh N P, Alba B M, Bose B, *et al*. OMP peptide signals initiate the envelope-stress response by activating DegS protease via relief of inhibition mediated by its PDZ domain. Cell, 2003, **113** (1): 61～71

26 Wilken C, Kitzing K, Kurzbauer R, *et al*. Crystal structure of the DegS stress sensor: how a PDZ domain recognizes misfolded protein and activates a protease. Cell, 2004, **117** (4): 483～494

27 Gupta S, Singh R, Datta P, *et al*. The C-terminal tail of presenilin regulates Omi/HtrA2. protease activity. J Biol Chem, 2004, **279** (44): 45844～45854

28 Murwantoko, Yano M, Ueta Y, *et al*. Binding of proteins to the PDZ domain regulates proteolytic activity of HtrA1 serine protease. Biochem J, 2004, **381** (3): 895～904

29 Hultgren S J, Jones C H, Normark S N. Bacterial adhesions and their assembly. In: Neidhardt F C ed. *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology. Washington, DC: ASM Press, 1996. 2730～2756

30 Hultgren SJ, Normark S, Abraham SN. Chaperone-assisted assembly and molecular architecture of adhesive pili. Annu Rev Microbiol, 1991, **45**: 383～415

多肽诱导的大肠杆菌 DegP (HtrA) 蛋白酶的构象变化 *

张雪峰 [1)] 郑益新 [2, 3)] 昌增益 [1, 2, 3)**]

([1)]清华大学生物科学与技术系，北京 100084；
[2)]北京大学蛋白质工程和植物基因工程国家重点实验室，北京 100871；
[3)]北京大学生命科学学院， 北京 100871)

摘要 具有分子伴侣和蛋白酶双重活性的大肠杆菌 DegP 蛋白，在热休克和其他应激条件下，对于降解和清除膜间质中变性或损伤的蛋白质起着十分重要的作用. 到目前为止，已有几种蛋白质被鉴定出是 DegP 的天然底物. 以前的研究表明，DegP 的体内底物之一，PapG 菌毛蛋白的羧基端多肽能够激活 DegP 的蛋白酶活性. 然而这种激活的机制及生理意义均未见报道. 用合成的 PapG 菌毛蛋白的羧基端多肽对这种激活的机制进行了初步研究. 结果表明，DegP 与多肽结合后发生了可检测的构象变化. 圆二色性光谱结果显示，结合多肽后 DegP 的二级结构和三级结构均发生了一定的变化. 凝胶排阻层析和动态光散射实验也揭示出 DegP 分子在一定程度上变小. 进一步实验表明，DegP 在多肽存在下，其疏水表面和催化位点均有所暴露. 荧光各向异性结果显示出 DegP 在结合多肽后其构象柔性降低. 对上述结果的意义进行了探讨.

关键词 DegP，构象变化，多肽，机制

学科分类号 Q512

---

*国家重点基础研究发展计划资助项目 (G1999075607) 和国家自然科学基金资助项目(30270289).

** 通讯联系人. 北京大学生命科学学院 183 信箱, 北京 100871
Tel: 010-62758822, Fax: 010-62751526, E-mail: changzy@pku.edu.cn

收稿日期：2005-09-22，接受日期：2005-11-01