Structure
Article

Characterization of the Structure and Function of *Escherichia coli* DegQ as a Representative of the DegQ-like Proteases of Bacterial HtrA Family Proteins

Xiao-chen Bai,[1,3,4] Xi-jiang Pan,[1,3,4] Xiao-jing Wang,[1,3] Yun-ying Ye,[1,3] Lei-fu Chang,[1,3] Dong Leng,[1,3] Jianlin Lei,[2,3] and Sen-Fang Sui[1,3,*]
[1]State Key Laboratory of Biomembrane and Membrane Biotechnology
[2]Ministry of Education Protein Science Laboratory
[3]Center for Structural Biology, School of Life Sciences
Tsinghua University, Beijing 100084, China
[4]These authors contributed equally to this work
*Correspondence: suisf@mail.tsinghua.edu.cn
DOI 10.1016/j.str.2011.06.013

SUMMARY

HtrA family proteins play a central role in protein quality control in the bacterial periplasmic space. DegQ-like proteases, a group of bacterial HtrA proteins, are characterized by a short LA loop as compared with DegP-like proteases, and are found in many bacterial species. As a representative of the DegQ-like proteases, we report that *Escherichia coli* DegQ exists in vivo primarily as a trimer (substrate-free) or dodecamer (substrate-containing). Biochemical analysis of DegQ dodecamers revealed that the major copurified protein substrate is OmpA. Importantly, wild-type DegQ exhibited a much lower proteolytic activity, and thus higher chaperone-like activity, than DegP. Furthermore, using cryo-electron microscopy we determined high-resolution structures of DegQ 12- and 24-mers in the presence of substrate, thus revealing the structural mechanism by which DegQ moderates its proteolytic activity.

INTRODUCTION

To a great extent, cell life depends on the function of proteins, most of which have marginally stable structures and are thus subjected to continuous quality control to keep them in a functional state. Failure of this process can lead to severe diseases (Sitia and Braakman, 2003). Molecular chaperones and proteases, both binding to unfolded or misfolded proteins, are two protein families that cells employ to perform such quality control processes (Lindquist and Craig, 1988; Wickner et al., 1999). DegP (also named protease Do and HtrA: high temperature requirement) was the first HtrA protease identified, and was found to be an essential protein for cell survival of *Escherichia coli* at elevated temperatures (Lipinska et al., 1988; Strauch and Beckwith, 1988). DegP homologous proteins, all of which comprise the HtrA family, play a central role in the quality control process, and have been found in nearly all organisms, including bacteria, plants, and mammals (Clausen et al., 2002; Pallen and Wren, 1997; Spiess et al., 1999).

In *E. coli*, DegP, DegS, and DegQ comprise the HtrA family proteins in the periplasmic space. In recent years, structural studies based on X-ray and cryo-electron microscopy (cryo-EM) have provided functional insights into both DegP and DegS (Jiang et al., 2008; Krojer et al., 2002, 2008b; Shen et al., 2009; Wilken et al., 2004). However, the structure and function of DegQ remains poorly understood. As typical HtrA members, these proteins contain a conserved N-terminal trypsin-like protease domain, and one (DegS) or two (DegP and DegQ) C-terminal PDZ domains (Krojer et al., 2002; Pallen and Wren, 1997; Wilken et al., 2004).

The crystal structures of DegS in both the peptide-free and peptide-bound states were resolved to be trimers (Wilken et al., 2004). In the peptide-free state, substrate binding and catalysis are prevented. After binding of the peptide to the PDZ domain, the proteolytic activity of DegS is activated (Sohn et al., 2007, 2009; Walsh et al., 2003; Wilken et al., 2004). Purified free DegP exists primarily as a resting hexamer, consisting of two trimers in a staggered face-to-face manner (Jomaa et al., 2007; Krojer et al., 2002). In the DegP hexamer, the L1 and L2 loops from each monomer twist into inactive conformations by the interaction with the LA loop from the opposite trimer (Krojer et al., 2002). Recent studies by Krojer et al. (2008b) and our group (Jiang et al., 2008; Shen et al., 2009) have shown that the DegP hexamer can assemble into high-order 12-, 15-, 18-, or 24-meric complex structures in the presence of substrates or on the membrane. In the larger oligomers, the LA loop is set free and the PDZ1 domain is locked into the proper position by the L3 loop, and this reposition of the L3 loop promote remodeling of the L1/L2 loops into functional catalytic sites (Krojer et al., 2010; Krojer et al., 2008b). Thus, forming larger oligomers is a critical step in initiating the proteolytic activity of DegP.

DegQ is another periplasmic serine protease in *E. coli* (Bass et al., 1996; Waller and Sauer, 1996). Unlike DegP, deletion of the *degQ* gene seems not to cause any defect in growth, indicating that DegQ is apparently not essential for cell survival, even at high temperatures (Farn and Roberts, 2004; Mo et al., 2006; Waller and Sauer, 1996). Transforming with a plasmid

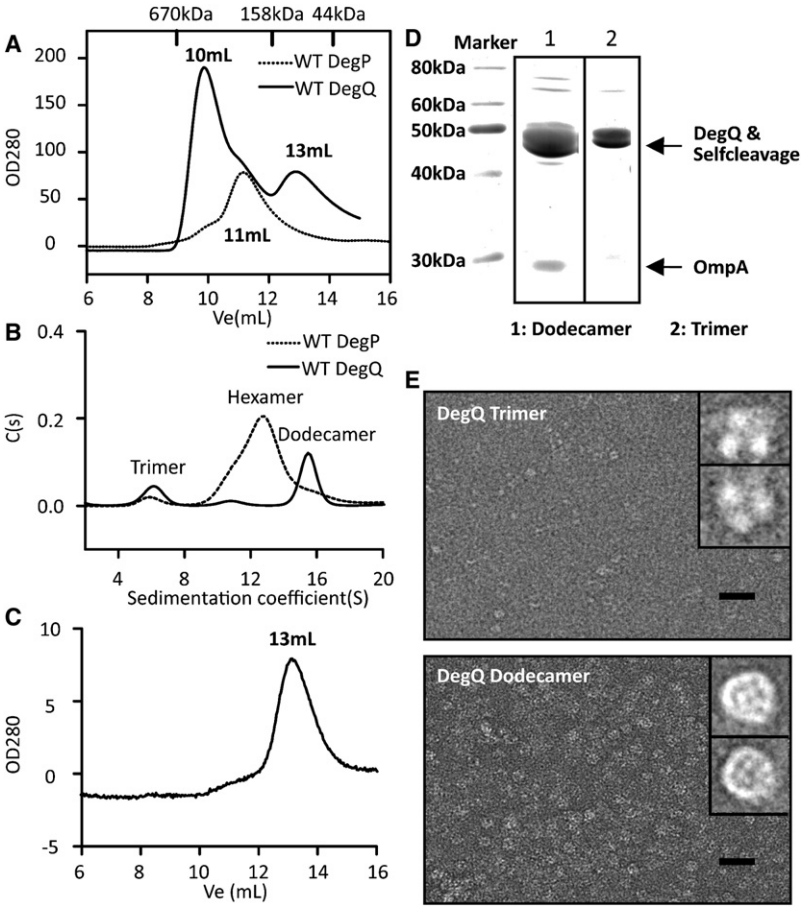


Figure 1. Purified DegQ Exists as Trimers, but Not Hexamers

(A) Size-exclusion chromatography (SEC) elution profiles for wild-type DegQ and wild-type DegP. DegQ was eluted at 10 ml and 13 ml, corresponding to dodecamers and trimers, respectively, and DegP at 11 ml, corresponding to hexamers. The protein weight markers are labeled above.

(B) Sedimentation velocity results for wild-type DegQ and DegP showing the different c(s) distributions.

(C) Rechromatography of the 13 ml fraction in (A) produced an identical peak.

(D) SDS-PAGE analysis of the 10 ml (lane 1) and 13 ml (lane 2) fractions. Mass spectrometry revealed that the additional band corresponded to OmpA.

(E) Electron micrographs of negative-stain samples from 13 ml (upper) and 10 ml (lower) fractions, showing the characteristics of DegQ trimers and dodecamers, respectively. The class-averages of particles are shown (inset). The box sizes of the class-averages are 14.4 nm (upper) and 20.6 nm (lower), respectively. Scale bars represent 50 nm.

See also Figure S1.

expressing the *degQ* gene rescues the phenotype of a *degP* null strain, suggesting that overexpressed DegQ can function as a substitute for DegP (Waller and Sauer, 1996). However, the inside story of DegQ remains incomplete. DegQ and DegP have similar amino acid sequences (58% identical), whereas the protease domain, and particularly the LA loop, exhibit less sequence homology. The LA loop is 40 amino acids long in DegP, but only 20 in DegQ. Based on phylogenetic analysis, recent studies (Kim and Kim, 2005; Onder et al., 2008) suggested that distinct DegP-like and DegQ-like periplasmic subfamilies exist, and many bacteria, unlike *E. coli*, have only one DegQ-like protease in the cell envelope. These DegQ-like proteases are characterized by short LA loops and are essential for cell survival under high envelope stress conditions including heat, chemicals, or pathogens, etc., all of which can result in protein inactivation and protein unfolding or misfolding (Kim and Kim, 2005; Onder et al., 2008). Thus, *E. coli* DegQ may be a model system for better understanding the molecular mechanisms involved in the function of other bacterial HtrA proteins.

In this study, we first undertook a biochemical and EM structural analysis to characterize the in vivo structural states of *E. coli* DegQ. Wild-type DegQ was found to exist primarily as a trimer and dodecamer in *E. coli* cell extract. Our data also demonstrated that dodecameric DegQ contained substrate, but trimeric DegQ did not. Biochemical analysis of the DegQ dodecamer complex revealed that the major copurified protein substrate was OmpA, a major component of the outer membrane of *E. coli*. Importantly, wild-type DegQ exhibited a much lower proteolytic activity than DegP. Furthermore, high-resolution structures of DegQ 12-mer/24-mer were obtained by cryo-EM with protease-deficient DegQ (S187A) in the presence of substrates, revealing the important role of PDZ2 domain in the assembly of 12- and 24-mers and providing a structural basis for regulation of the proteolytic activity of DegQ.

RESULTS

Wild-Type DegQ Exists Primarily as a Trimer and Dodecamer

X-ray crystallography has shown that DegP exists in the resting state as a hexamer (Krojer et al., 2002). Previous studies have shown that the LA loops are essential for maintenance of this hexamer (Jomaa et al., 2007; Krojer et al., 2010; Sobiecka-Szkatula et al., 2009). DegQ has a much shorter LA loop, suggesting that the oligomeric states of DegQ and DegP may differ. To determine the in vivo oligomeric state of DegQ, size-exclusion chromatography (SEC), in combination with sedimentation velocity (SV) measurement, was performed on *E. coli* cell extracts. Wild-type DegP was used as a control. The SEC profile of wild-type DegP exhibited a major peak at elution volume 11.0 ml, corresponding to the DegP hexamer (Figure 1A), which was consistent with previous reports (Jiang et al., 2008; Jomaa et al., 2007). Unexpectedly, wild-type DegQ was isolated mainly in two fractions at elution volumes 10.0 and 13.0 ml, corresponding to molecular masses of approximately 600 (12-mer) and 150 kDa (3-mer), respectively (Figure 1A). The existence of the 10 ml dodecameric DegQ fraction was consistent with the cross-linking result of Kolmar et al. (1996). Identical results

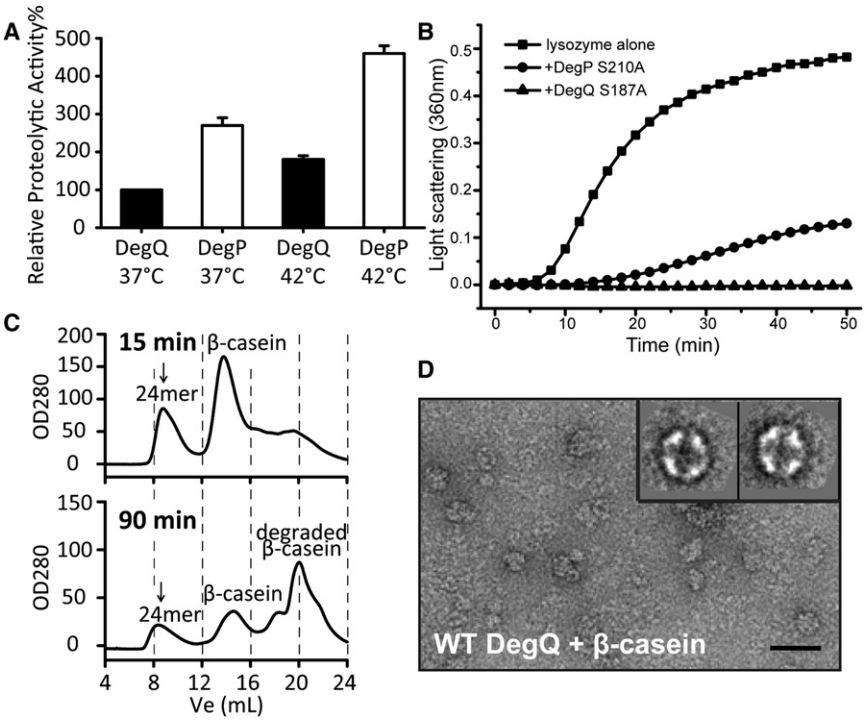


Figure 2. DegQ Trimers Exhibit Lower Proteolytic Activity and Higher Chaperone-like Activity

(A) Proteolytic activity of DegQ and DegP (0.2 mg/ml) at 37°C and 42°C using resorunfin-labeled casein as the substrate. The relative protease activities were calculated by considering the activity of DegQ at 37°C as 100%. The histograms of DegP are shown in white. Error bars represent standard deviations (SD) of repeated measurements.

(B) The chaperone-like activity of DegQ (S187A) and DegP (S210A) protected DTT-denatured lysozyme against aggregation. The light-scattering values for the incubated samples were recorded at 360 nm. Error bars represent SD of repeated measurements.

(C) SEC elution profile of wild-type DegQ and β-casein after varying incubation periods. Large complexes were formed whereas DegQ was degrading β-casein. The peak of β-casein and degraded β-casein are labeled above the profile.

(D) An electron micrographs of negative-stain wild-type DegQ/β-casein complexes from the fractions indicated in (C) by the arrows. Class-averages are shown (inset). The scale bar represents 50 nm.

See also Figure S2.

were obtained by SV analysis (Figure 1B). In the DegP sample, there were two well-resolved species having sedimentation coefficients of approximately 6S and 13S, corresponding to trimers and hexamers (Figure 1B). This may be due to small amount of disassociation of DegP hexamers as revealed before (Jiang et al., 2008; Krojer et al., 2008b). In the case of DegQ, two well-resolved species were also detected, but with sedimentation coefficients of ~6S and 16S (Figure 1B). The first peak appeared in the same position as DegP trimers, suggesting that they were DegQ trimers. The second peak, however, was at a point greater than DegP hexamers, implying a dodecameric state of DegQ (Figure 1B). According to peak heights, the amount of DegQ dodecamers was higher than trimers, in agreement with the SEC results (Figures 1A and 1B). The 13.0 ml trimeric DegQ fraction was then reexamined by SEC and no larger species were detected, ruling out the possibility of self-assembly of DegQ in solution (Figure 1C). SDS-PAGE revealed that the 10.0 ml fractions (12-mer) contained an additional protein band at 30 kDa in addition to DegQ (Figure 1D), which was further identified to be OmpA by mass spectrometry (see Figure S1 available online). In SDS-PAGE, folded OmpA migrates at 30 kDa (Figure 1D), whereas unfolded OmpA migrates at 35 kDa (Schweizer et al., 1978). The SDS-PAGE result suggested that the OmpA encapsulated by DegQ is likely present in folded state.

To further investigate the oligomeric state of DegQ, the 13 ml and 10 ml fractions were collected and examined by negative-stain EM. Particle size was estimated to be 10–12 nm in the 13 ml fraction (Figure 1E, upper), consistent with the size of a DegP trimer (Krojer et al., 2002). Reference-free classification of the particles indicated clear 3-fold symmetry (Figure 1E, upper), which resembled the projection of the top view of a DegP trimer (Krojer et al., 2002). In contrast to the DegQ trimers, particles composed of both DegQ and OmpA were much larger (~16 nm diameter; Figure 1E, lower), with dimension and shape similar to the DegP dodecamer (Jiang et al., 2008). Indeed, the class averages of the complexes were similar to the projections of the DegP dodecamer (Figure 1E, lower) (Jiang et al., 2008).

The different oligomeric states of DegP and DegQ in vivo strongly suggest that DegQ may exhibit different proteolytic and chaperone-like activities from DegP in the cell. Thus, the trimeric DegQ fraction was subsequently subjected to proteolytic and chaperone assays.

Wild-Type DegQ Exhibits Lower Proteolytic and Higher Chaperone-like Activities than DegP

The protease activity of DegQ was assessed by incubating resorunfin-labeled casein with the trimeric form of the SEC fraction of wild-type DegQ. Unexpectedly, wild-type DegQ trimers (0.2 mg/ml) exhibited much weaker proteolytic activity than DegP hexamers (0.2 mg/ml) at both 37 and 42°C (Figure 2A). These data were further confirmed by SDS-PAGE using two substrates, β-casein or denatured lysozyme. DegP at different concentrations degraded β-casein much quickly than DegQ (Figure S2C). In 60 min, nearly all β-casein was completely degraded by DegP, whereas only a small fraction of β-casein was degraded by the same amount of DegQ (Figure S2C). In comparison with β-casein, DegP degraded denatured lysozyme at a relatively low rate, however, it still exhibited much greater degradation capability than DegQ (Figure S2D).

We hypothesized that this lower protease activity of DegQ would be accompanied by a higher chaperone-like activity compared with DegP. To verify this, we compared the chaperone-like activity of DegQ (S187A) with that of DegP (S210A) at 37°C. As expected, DegQ (S187A) exhibited stronger

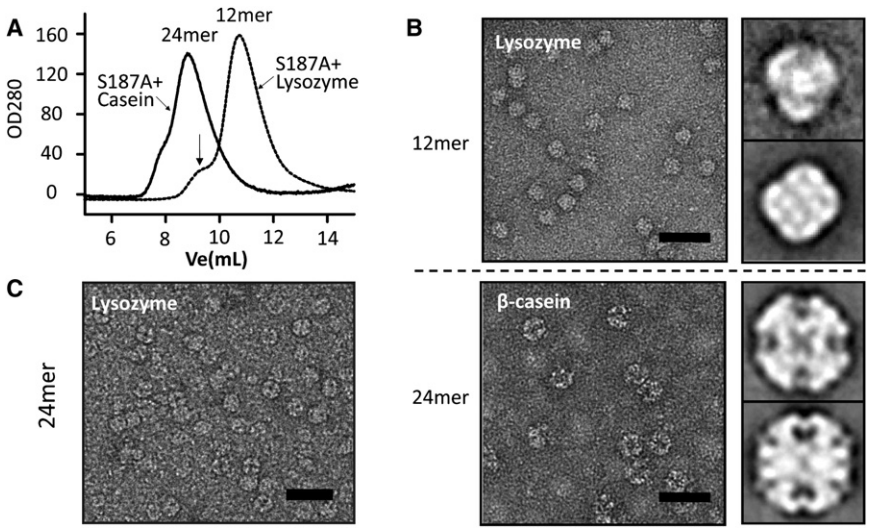


Figure 3. The Oligomerization of DegQ Trimer into 12-/24-mer in the Presence of Different Substrates

(A) SEC elution profiles for DegQ (S187A) in the presence of denatured lysozyme or β-casein.

(B) Electron micrographs of negative-stain DegQ (S187A)/lysozyme complexes 12-mer (upper) and DegQ (S187A)/β-casein complexes 24-mer (lower). Class-averages for the two different types of complexes are shown on the right, respectively. Scale bar represents 50 nm.

(C) An electron micrograph of negative-stain DegQ (S187A)/lysozyme complexes from the fraction indicated in (A) by the arrow, showing the characteristic of DegQ 24-mer. Scale bar represents 50 nm.

See also Figure S3.

chaperone-like activity toward denatured lysozyme than DegP (S210A) at 37°C (Figure 2B). In particular, the aggregation of denatured lysozyme was entirely suppressed by DegQ from the beginning, whereas it was only partially suppressed by DegP. In parallel, we tested the ability of DegP to prevent thermal-induced aggregation of citrate synthase (CS) at 43°C. Similar to the result from the lysozyme assay, DegQ S187A had significantly stronger protecting effect against the thermal-induced aggregation of CS than DegP S210A in solution (Figure S2A). Further enzymatic activity assay of CS in the two systems proved that DegQ S187A has stronger protection capacity against the denatured protein than DegP S210A in solution (Figure S2B).

Because DegQ exhibited a much lower rate of degradation, we next attempted to capture the intermediate state of DegQ during degradation. After incubating excess β-casein with wild-type DegQ trimers at 37°C for 15 min, the elution volume shifted entirely from 13 ml to 8.8 ml on a SEC (Figure 2C), indicating that most of the wild-type DegQ trimers had assembled into larger oligomers once degradation begun. Examined by negative-stain EM and reference-free classification, the 8.8 ml fraction clearly contained particles of 24-mers, but not trimers (Figure 2D). Such large complexes represented an active protease form of DegQ was supported by the fact: after a long time of incubation (90 min), the peak of β-casein decreased to a great extent, meanwhile, the peak of degraded β-casein increased (Figure 2C).

To investigate the causes for DegQ's higher chaperone-like and lower proteolytic activity than DegP, we incubated DegQ with a variety of substrates to observe its functional oligomeric state and resolved the high-resolution structure of DegQ/substrate assemblies by cryo-EM.

Binding of Substrate by DegQ Results in Formation of 12-/24-mers

Conversion from hexamers to 12-/24-mers in the present of substrate is a crucial step in activation of DegP protease activity (Jiang et al., 2008; Krojer et al., 2008b). Large DegQ oligomers, namely 12- or 24-mers, were also observed by SEC and EM when protease-deficient DegQ (S187A) trimers were incubated with denatured lysozyme or β-casein (Figures 3A and 3B; Figure S3A). Additionally, a proportion of DegQ formed 24-mers with excess amount of denatured lysozyme (Figure 3C). To further characterize the structure of DegQ oligomers, we obtained high-resolution structures of DegQ (S187A) 12- and 24-mers from a total of 24,604 and 25,783 particles, respectively, using cryo-EM and single-particle reconstruction (Figure 4A; Figures S3A and S3B). The resolutions of 12- and 24-mers achieved are 8.2 and 6.5 Å, respectively, based on FSC 0.5 criteria (Figure S3C). Inspection of the density map and atomic model fitting suggests that the actual resolution is ~8 Å for DegQ 24-mer and ~10 Å for DegQ 12-mer, because α helices are only partly resolved. Both types of cage-like structure consist of similar units with characteristics of a DegP trimer (Figures 4A; Figure S4B). Four and eight DegQ trimers assemble via lateral interactions to form a 12-mer (16 nm in diameter) and 24-mer (19 nm in diameter), respectively (Figure S3B). The β-casein molecules located in the cavity were not resolvable in the DegQ 24-mer, because the disordered substrates were averaged out (Figure S4A, right). However, extra densities were observed in the cavity of DegQ 12-mers, although the tetrahedral symmetry was imposed during the reconstruction process (Figure S4A, left), presumably as a result of the relatively ordered substrate and small inner space of the 12-mer structure.

The monomer extracted from the DegP 24-mer crystal structure (Protein Data Bank [PDB] ID: 3CS0) (Krojer et al., 2008b) was fitted as a rigid body, into the EM densities of the DegQ 12- and 24-mers. However, the protease and PDZ1/PDZ2 domains could not be fitted into the density maps simultaneously. Thus, the three domains had to be fitted into the density maps separately, which showed good agreement between the atomic models and the density maps (Figure 4B). The structural features of the protease domain were clearly observed in the DegQ 24-mer density map. For example, the α helices (αA and αE) from DegP fitted well into the protease domain of DegQ 24-mer (Figure 4C, left). However, the L2/L3 loop regions of DegP largely protruded out from the DegQ density map (Figure 4C,

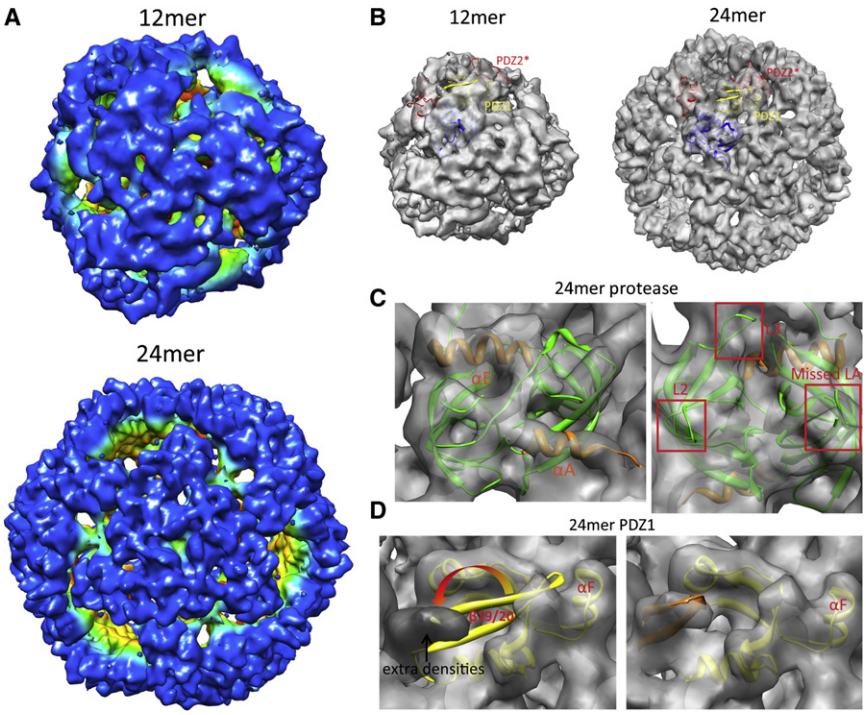


Figure 4. Cryo-EM Models of DegQ Oligomers and Atomic Model Fitting

(A) Radially colored surface view for 3D reconstruction of DegQ (S187A)/lysozyme complexes 12-mer (upper) and DegQ (S187A)/β-casein complexes 24-mer (lower).

(B) Atomic model of a DegP 24-mer (PDB ID: 3CS0) docked into DegQ 12-/24-mers semi-transparent maps (the PDZ1 domain and the PDZ2 domain from a neighboring trimer are labeled as PDZ1 and PDZ2*, respectively). The protease, PDZ1, and PDZ2 domains are shown in blue, yellow, and red, respectively.

(C) Representative local fitting results of the DegQ 24-mer. The αE and αA helices are shown in orange (left). Local differences between DegQ and DegP near the catalytic center are indicated by red boxes (right).

(D) Atomic model of the PDZ1 domain of a DegP 24-mer (PDB ID: 3CS0) docked into a DegQ 24-mer. The overall fit is good (the helices αF is labeled), with the exception of β19/20 (left). The color of the extra densities is darkened and manual adjustment is indicated by the arrow (left). β19/20 after modification is shown in orange (right).

See also Figure S4.

right), indicating a structural difference in these regions that will be discussed in the following section.

Some PDZ1 domain α helices were resolved in the DegQ 24-mer density map (e.g., the αF from DegP fitted well) (Figure 4D) allowing us to precisely dock the atomic model. The overall fit was good, with the exception of β strands β19/20 from DegP, which protruded outside of the DegQ map (Figure 4D, left). As expected, nearby EM density was unfilled, with a size and shape characteristic of β19/20 (Figure 4D, left), hinting that β19/20 could undergo a position change compared with that of DegP. An improved local fitting was obtained by bending a nonconserved linker, which is connected to β19/20 (Figure 4D, right; Figure S4C).

The PDZ2 Domain, Rather Than the PDZ1 Domain, Plays an Important Role in Assembly of Different Sizes of Oligomer

After precisely positioning all three domains into one trimer and another PDZ2 domain into the adjacent trimer, it was clear that the DegQ 12- or 24-mer was formed by a similar PDZ1-PDZ2* interaction between neighboring trimers as in DegP assemblies (Figure 4B). In more detail, αF from the PDZ1 domain and β25/26 from the PDZ2* domain come together to form the PDZ1-PDZ2* interface (Figure 5A). Such a critical intertrimer PDZ1-PDZ2* interaction pattern was characteristic in all DegP/Q 12- and 24-mers (Figure 5A) (Jiang et al., 2008; Krojer et al., 2008b).

DegQ and DegP both can form both 12- and 24-mers. However, how PDZ domains reorient during conversion from 12- to 24-mer remains unknown. Based on the atomic model fitting of EM maps of DegQ 12-/24-mers, we aligned the protease or PDZ1 domains together to explore the orientation change of PDZ1/2 domains between the 12- and 24-mers. Relative to the DegQ 24-mer, the PDZ1 domain of the 12-mer rotated $\leq 7^{\circ}$ around the protease domain (Figure 5B); in contrast, the PDZ2 domain underwent a rotation of $\sim 35^{\circ}$ around the PDZ1 domain (Figure 5C). In light of the orientation change, the position of the PDZ1 domain appears relatively fixed after conversion from a 24-mer to a 12-mer (Figure 5B), consistent with the PDZ1 domain of DegP being locked by the L3 loop after oligomerization (Krojer et al., 2010). In DegP, the PDZ1 and protease domains collaborate during degradation (Krojer et al., 2008a), indicating that the proper distance between the PDZ1 and protease domains is important for degradation. We compared DegQ 12- and 24-mers with DegP 24-mer and found that the distances between the substrate binding site in PDZ1 domain and the catalytic center in protease domain were all the same among the three different oligomers (Figure 5B). This result further demonstrated that the position of PDZ1 domain remains almost unchanged in order to eliminate the unfolded proteins efficiently, even if the oligomeric state has altered. Thus, the PDZ2 domain, rather than the PDZ1 domain, is mainly responsible in the assembly of different sizes of oligomer.

A Groove-like Structure May Mediate DegQ Proteolytic Activity

As mentioned above, the proteolytic activity of DegQ is much weaker than that of DegP. We have ruled out the possibility that this is due to a different activation mechanism; thus, the catalytic center of DegQ may undergo a conformational change. To test this, we compared the protease domains of the DegP and DegQ 24-mers. Interestingly, a groove-like structure was observed in the center of the DegQ protease domain (Figure 6A). After fitting, the residue S210 of DegP, one of the catalytic triad

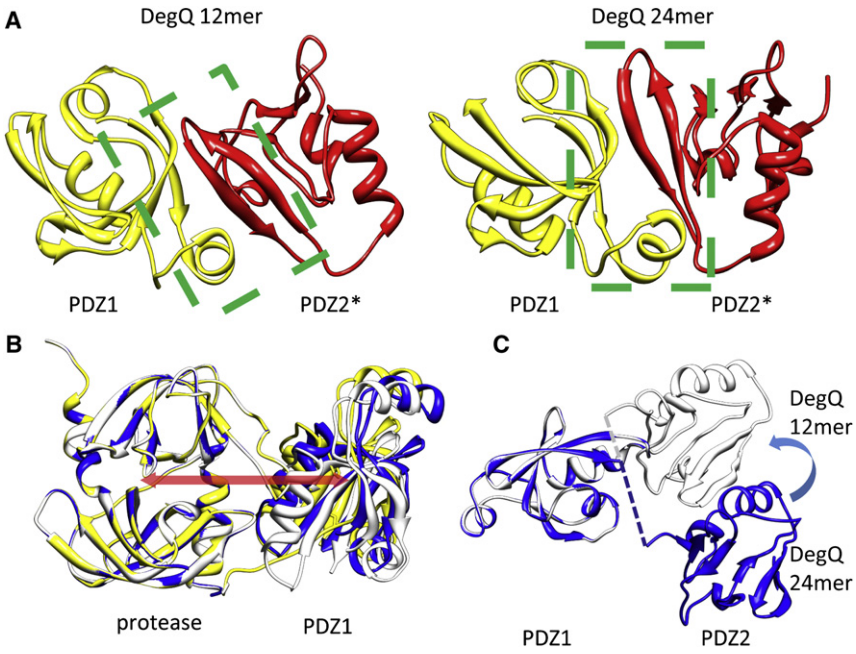


Figure 5. The PDZ1-PDZ2* Interaction and the Orientation Change of PDZ Domains between 12- and 24-mers

(A) A similar PDZ1-PDZ2* interaction between DegQ 12- (left) and 24-mer (right). PDZ1 and PDZ2 are shown in yellow and red, respectively. The interfaces formed by αF and β25/26 are indicated by green boxes.

(B) The relatively fixed position of PDZ1 among DegP 24-, DegQ 12-, and 24-mers relative to the protease domain. The DegP 24-, DegQ 12-, and 24-mers are shown in yellow, white, and blue, respectively. The protease domains of three different oligomers are aligned together. The distance between the catalytic center and the substrate binding site of PDZ1 domain is indicated by the arrow.

(C) The orientation change of PDZ2 between DegQ 12- and 24-mers relative to the PDZ1 domain is indicated by the arrow. The DegQ 12- and 24-mers are shown in white and blue, respectively. The PDZ1 domains of two different oligomers are aligned together.

was located at the bottom of the groove-like structure in DegQ (Figure 6A). For comparison, the atomic model of the DegP 24-mer was converted to electron density and filtered to a resolution similar to DegQ 24-mer (Figure 6B, right). The DegP catalytic center was located on the surface region with a feature of wide-open pocket (Figure 6B, right), which was remarkably different from the groove-like structure of DegQ (Figure 6B, left). We suggest that the existence of a narrow groove may affect substrate access to the catalytic center. Indeed, if we tried to dock the substrate peptide, derived from the crystal structure of the DegP 24-mer (PDB ID: 3MH7) (Krojer et al., 2010), it interfered with the side wall of the groove (Figure 6C), supporting our hypothesis.

To further confirm our data, the crystal structures of DegS in both the active and inactive states (PDB ID: 1SOT, 1SOZ) (Wilken et al., 2004) were fitted into our EM density map (Figure 6D). The atomic model of DegS in the inactive state resulted in a better fit compared to DegS in the active state (Figure 6D). Obviously, the L2/L3 loops of active DegS largely protruded out from the DegQ map. The correlation coefficients (calculated by Situs) (Wriggers et al., 1999) were 0.77 and 0.83 for active and inactive DegS, respectively, indicating that DegQ in its functional state resembles inactive DegS. This result suggests that the proteolytic activity of DegQ may not be completely activated via substrate-induced oligomerization.

Moreover, two differences in the docking results of the active center were evident. First, the L2/L3 loops of DegP protruded from the DegQ density map, and second, a large volume of the side wall of the groove-like structure was unfilled (Figure 4C, right). Thus, DegQ L2/L3 loops must undergo a conformational change to achieve an acceptable fit. To quantitatively describe the orientation change of the loops based on the high-resolution cryo-EM map, the MODELER and molecular dynamics flexible fitting (MDFF) methods (Sali et al., 1995; Trabuco et al., 2008) were used to simulate the atomic model of DegQ (Figure 6E). Subsequently, the two atomic models (DegQ by cryo-EM-based simulation and DegP by X-ray crystallography) were aligned, showing that most of the atomic structure overlapped perfectly, with the exception of the L2/L3 loops (Figure 6F). The L2 loop of DegQ was tilted ~30° toward the catalytic center relative to the activated DegP (Figure 6F), similar to the inactivated DegP to a certain extent. Meanwhile, the L3 loop was tilted ~30° toward the PDZ1 domain (Figure 6F). Because the active-site L2/L3 loops are known to play a crucial role in regulating the protease activity of various oligomers of DegP, any distortion of them in DegQ will likely affect the proteolytic activity.

DISCUSSION

Despite the generally high homology of the protease domain of the HtrA family, the Q-linker, which is also called LA loop in DegP and DegQ of *E. coli*, exhibits low sequence homology. Recent studies (Krojer et al., 2010) indicated that the LA loop plays a key role in regulating the function of DegP during heat stress conditions. DegQ is characterized by a short LA loop in its protease domain. Although the function of DegQ in *E. coli* remains unknown, it is of interest because many bacteria, unlike *E. coli,* lack DegP. These organisms contain only one DegQ-like protease that is also characteristic with a short LA loop (Kim and Kim, 2005; Onder et al., 2008). Thus, study of the structure and function of *E. coli* DegQ may assist in understanding the functional mechanisms of DegQ-like proteases that are possessed by many bacteria.

At low temperatures, free DegP exists as stable hexamers. In this form proteolytic activity is greatly suppressed to prevent mistaken degradation of proteins (Spiess et al., 1999). In contrast, free DegQ trimers are incapable of inhibiting their proteolytic activity, which would seem to be harmful to the cell. To make up for this defect, DegQ has low proteolytic, but high chaperone-like activity. These results indicate that free DegQ

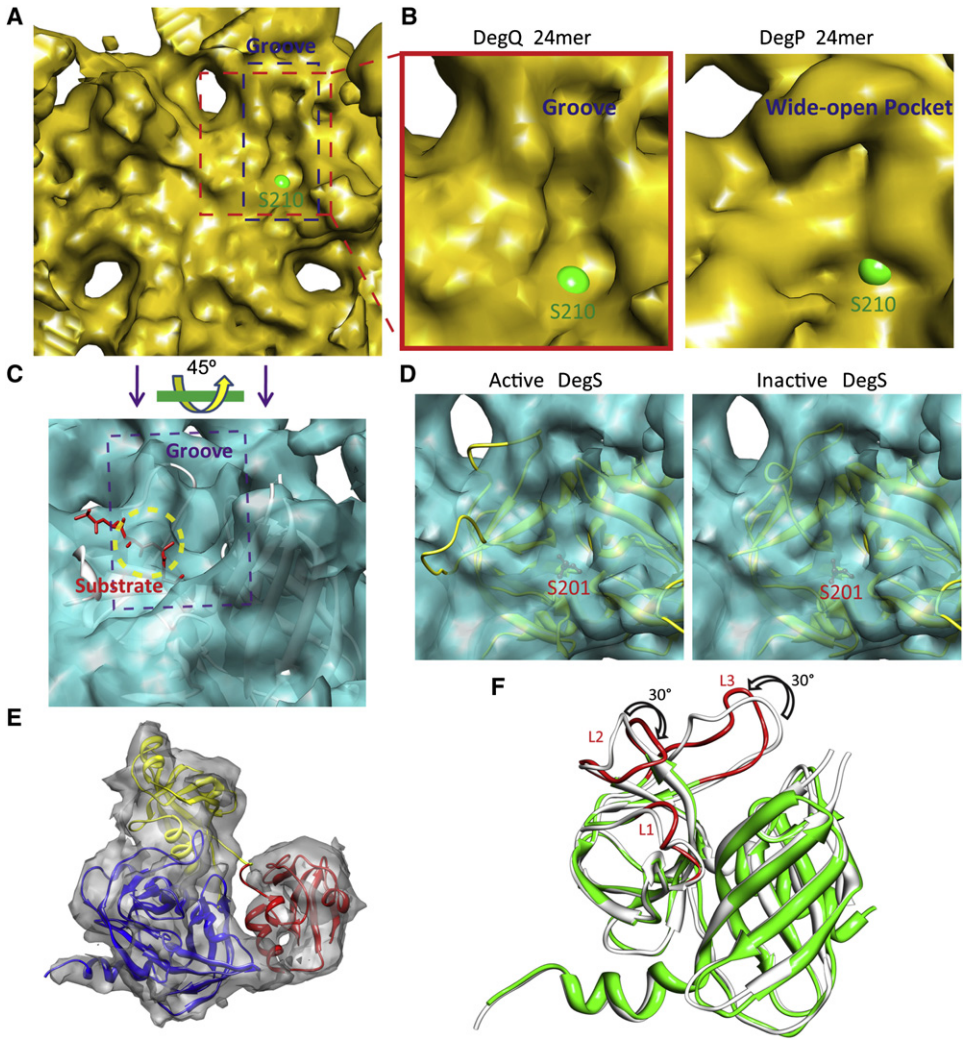


Figure 6. A Groove-like Structure Partially Blocks the Entrance to the Catalytic Center and Affects the Proteolytic Activity of DegQ

(A) A groove-like structure near the catalytic center in the EM map of a DegQ 24-mer, indicated by a blue box. S210, one of the catalytic triad in DegP, is labeled in green.

(B) Structural comparison of the catalytic center of a DegQ 24-mer (left) and a DegP 24-mer (right). The electron map of DegP (right) was converted from the crystal structure of the DegP 24-mer (PDB ID: 3CS0).

(C) An atomic model of a DegP 24-mer in complex with substrate (PDB ID: 3MH7) docked into the EM map of a DegQ 24-mer. The substrate is blocked by the sidewall of the groove in DegQ (the overlapping area indicated by a yellow circle).

(D) Atomic models of DegS in both the active (PDB ID: 1SOZ) and inactive (PDB ID: 1SOT) states, docked into the EM map of a DegQ 24-mer. Inactive DegS provided a better fit (right). S201, one of the catalytic triad in DegS, is labeled in red.

(E) Atomic model of DegQ simulated by MODELER and MDFF based on a high resolution EM map. A monomer extracted from the EM map of a DegQ 24-mer is shown in white. The protease and PDZ1/2 domains of DegQ generated by cryo-EM based simulation are shown in blue, yellow, and red, respectively.

(F) Structural comparison of the protease domains of DegQ and DegP. Atomic models of DegQ and DegP are aligned together, and are colored in green and white, respectively. The L1, L2, and L3 loops of DegQ are colored in red. The conformational changes of the L2 and L3 loops between DegQ and DegP are indicated by the arrows.

trimers may serve mainly as a chaperone and rapidly bind denatured or partially folded substrates to protect rather than degrade them. This hypothesis is supported by another observation: many stable wild-type DegQ/OmpA complexes were obtained during the purification process. In contrast, it was difficult to maintain oligomers of wild-type DegP with OMPs in vivo (Jiang et al., 2008), because the larger oligomers were in a transient state and immediately reformed to hexamers after degradation. When protease-deficient DegP (S210A) was substituted for wild-type DegP, lots of oligomers in complex with OMPs could be purified from extracts (Krojer et al., 2008b), supporting our hypothesis.

In DegP, hexamers (resting state) can disassemble to trimers (ready state) more easily at high temperature (Jiang et al., 2008; Krojer et al., 2010, 2008b), explaining why its proteolytic activity is improved with the increase of temperature. DegQ exists primarily as trimers in the native state; however, the proteolytic activity of DegQ also shows temperature-sensitivity

to a certain extent (Figure 2A). This result suggests that some structural motifs in DegQ, such as active-site loops, may involve in the thermoregulation of proteolytic activity. Previous study has shown that a helical lid from *Thermotoga maritima* (Tm) HtrA that covers the active site is lifted up to expose the catalytic sites at elevated temperatures (Kim et al., 2008), further supporting our hypothesis.

The overall conformation of DegQ is similar to that of DegP. However, the high-resolution model shows local conformational features unique to each, such as β19/20 in the PDZ1 domain. DegQ trimers oligomerize to form 12- and 24-mers on binding substrate proteins. This activation mechanism, homo-oligomerization, is identical to that of DegP (Jiang et al., 2008; Krojer et al., 2008b). Our data thus support the result that these oligomers assemble by means of an interaction between the PDZ1 and PDZ2 domains. Previous studies have not provided details on how a series of high-order complexes are formed by the same structural unit. In this study, the high-resolution structures of DegQ 12- and 24-mers were resolved. When the two structures are compared, the orientation and position of the PDZ2 domains need extensive modification via bending of the flexible linkers. This indicates how DegQ trimers form different cage-like structures to enclose substrates with different sizes. In DegP and DegS, the L3 loop transduces the signal from the PDZ domain to the protease domain and reconfigures the L1/L2 loops into a functional site (Krojer et al., 2010; Walsh et al., 2003; Wilken et al., 2004). In our cryo-EM structure of DegQ, all L2/L3 loops underwent a conformational change compared with DegP, giving rise to formation of a groove-like structure. As a result, substrate entrance to the active site is partially blocked by the groove and thus degradation may be partially inhibited, explaining the lower proteolytic activity of DegQ. This result further corroborates the hypothesis that the primary role of DegQ in the cell is as a chaperone. More evidence, using techniques such as X-ray crystallography, is required to fully understand the role of DegQ.

During heat shock, DegP is induced and its proteolytic activity is upregulated to remove misfolded proteins as quickly as possible and thus protect cells. Under normal conditions, the function of DegP is partially inhibited and DegQ instead may play major roles in the protein quality control. The discussion by Önder et al. (2008) gives us salient cues for defining the role of DegQ in *E. coli*. The optimal growth temperature for enteric bacteria such as *E. coli* is ~37°C. Önder et al. (2008) indicated that DegQ, a second protease, might reflect an adaptive response of bacteria to higher physiological growth temperatures. Enteric bacteria seem to require a DegQ-like protease activity continuously and are able to overproduce a DegP-like protease during heat shock. In our model, when a small quantity of misfolded protein was present, DegQ immediately sequestered and protected them, thus delaying the occurrence of heat shock, frequent occurrence of which would be harmful to the cell. That is, DegQ serves as a "buffer pool" and so complements the activity of DegP.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents

β-casein was purchased from Sigma, resorufin-labeled casein was from Roche, protein assay kit was from Pierce, lysozyme was from Amersco, and DTT was from Merck. The other chemicals were reagent grade and purchased from commercial source.

Plasmid Construction

The wild-type *degQ* gene carried on the pTdeg plasmid was amplified by PCR and inserted into the pET-28a expression plasmid vector, after being cleaved with *Nco*I and *Xho*I restriction enzymes to generate pET-28a-degQ. A 6× Histidine tag was added to the C terminus of the DegQ protein. Overlapping strategy was adopted to generate DegQ mutant constructs.

Protein Expression and Purification

Wild-type and respective mutants of DegP and DegQ were expressed and purified as described with minor modification (Jiang et al., 2008; Shen et al., 2009). All proteins were suspended in PBS buffer containing 40 mM Na_2HPO_4, 10 mM $NaHPO_4$, 25 mM NaCl (pH 7.6). Protein concentration was measured using Pierce protein assay kit.

Size Exclusion Chromatography

Gel filtration chromatography was performed on a Superdex 200 column (GE Healthcare Life Sciences) equilibrated in buffer (40 mM Na_2HPO_4, 10 mM $NaHPO_4$, 25 mM NaCl, pH7.6) at 4°C. Protein samples (1 ml) were applied to the column at concentrations ranging between 0.5 and 2 mg/ml at a flow rate of 0.4 ml/min.

Sedimentation Velocity Analysis

Sedimentation velocity measurements were performed on the Proteomelab XL-I analytical ultracentrifuge (Beckman Coulter, Fullerton, CA) equipped with a three channel An-60 Ti rotor. The protein samples (380 μl) at concentration 0.6 mg/ml and corresponding buffer (400 μl) were loaded in pair into the double sector quartz cell and run at 42,000 × rpm at 20°C. Data were collected at wavelength of 280 nm in a continuous scan mode with scanning spaces of 30 s. Sedimentation coefficient distribution (c(s)) was calculated using the program SEDFIT.

Mass Spectrometry

To identify copurified protein substrates, protein band were excised from the SDS-PAGE gel and digested with trypsin. The digested sample was loaded onto a homemade C18 column (100 mm × 100 μm) packed with Sunchrom packing material (SP-120-3-ODS-A, 3 μm) and followed by nano-LC-ESI-MS/MS analysis. The LTQ mass spectrometer was operated in the data-dependent mode in which the initial MS scan recorded the mass-to-charge (m/Z) ratios of ions over the mass range from 350–1700 Da. The five most abundant ions were automatically selected for subsequent collision-activated dissociation. All MS data were searched against *E. coli* Database downloaded from the NCBI database using the SEQUEST program (Thermo, USA).

Proteolytic Activity Assay

Resorufin-labeled casein was used as the substrate protein to quantitatively determine the protease activity of DegQ and DegP. Mixtures of 200 μl resorufin-labeled casein (0.4% [w/v] in PBS buffer) and 200 μl buffer (0.2 M Tris-HCl, pH 7.8, 0.02 M $CaCl_2$) were added into 400 μl protein solution containing a certain amount of DegQ or DegP (0.2 mg/ml), and incubated at 37°C or 42°C. Every 15 min, a 40-μl sample was removed and added into 96 μl of 5% TCA to terminate the reaction and incubated for 10 min at 37°C. Then the mixtures were centrifuged for 5 min at 20,000 g. An 80-μl sample of the supernatant was mixed with 120 μl assay buffer (0.5 M Tris-HCl, pH 8.8) and the absorbance at 574 nm was immediately measured. The protease activity was calculated from the slope of the linear range of the absorbance curve (Jiang et al., 2008; Jomaa et al., 2007).

To characterize the protease activity against other substrates, β-casein or DTT denatured lysozyme were mixed with a certain amount of DegQ trimer or DegP hexamer and placed at 37°C. All samples were subjected to SDS-PAGE, and quantitation of protein bands of lysozyme was analyzed densitometrically by using TotalLab (version 2.01) program (Shen et al., 2009).

Chaperone-like Activity Assay

Mixtures (1 ml) of the DegQ or DegP protein (0.09 mg/ml) and lysozyme (0.1 mg/ml), containing 50 mM Na_2HPO_4 -NaH_2PO_4, 20 mM DTT, pH7.6,

were incubated at 37°C. Aggregation of lysozyme was monitored by measuring the light absorption at 360 nm with a spectrophotometer (Jiang et al., 2008; Shen et al., 2009; Skorko-Glonek et al., 2007).

Light scattering and enzymatic activity assays of CS (Sigma) were carried out as described (Buchner et al., 1998). To determine the aggregation kinetics, light scattering was measured at 500 nm under time scan mode in an FL-4500 fluorescence spectrophotometer (Hitachi) using an external bath circulator (Thermo). Stock solutions of 0.435 mg/ml CS was diluted 100-fold with stirring into 50 mM HEPES-KOH (pH 7.5), which was preincubated at 25°C. The inactivation reaction was started by plunging the solution into prewarmed curettes at 43°C. DegQ S187A or DegP S210A (final concentration, 0.01 mg/ml) were added before CS at 25°C. At the indicated time points, an aliquot of 10 μl was withdrawn and added to 190 μl assay buffer (50 mM Tris-HCL [pH 7.5], 2 mM EDTA, 0.5 mM oxalacetic acid, 0.3 mM acetyl-coenzyme A, 0.1 mM DTNB) to determine the remaining CS activity based on the first step of the citric acid cycle. In brief, the CoA formed in this assay stoichiometrically reduces the Ellman's reagent dithio-1, 4-nitrobenzoic acid (DTNB), resulting in an increased absorbance at 412 nm. The highest specific activity value at the starting point was considered as 100%, and the calculated specific activities at other time points were expressed as the percentage of this value. Each experiment was performed three times, and the standard deviation was calculated for each reaction.

Preparation of the DegQ-Substrate Complexes

The preparation of the DegQ-substrate complexes was carried out as described (Jiang et al., 2008). Briefly, the purified DegQ trimers were added to the unfolded substrate proteins (β-casein or lysozyme) in a molar ratio of 1:2 and incubated at 37°C for 30 min. DTT was added to lysozyme at the final concentration of 20 mM to unfold it.

EM and Image Processing

Negative-stain samples were prepared as described (Adair and Yeager, 2007). Briefly, after being washed with buffer two times, the grids were washed with buffers twice and then negative-stain with 1% uranyl acetate. The negative-stain samples were imaged on an FEI F20 TEM operated at 200 kV and a nominal magnification of 50,000×.

Fresh DegQ/substrate complexes were rapidly frozen by plunging them into liquid ethane using Vitrobot Mark IV (FEI Company) and stored in liquid nitrogen. The cryo samples were imaged on an FEI Titan Krios TEM equipped with an Eagle 4k × 4k CCD camera, operated at 300 kV with the dosage of 20 $e^{-}/Å^{2}$ at a nominal magnification of 59,000× . The defocus was set to between −1.5 and −3 μm, and the final pixel size is 1.46 Å/pixel.

Electron micrographs were evaluated by fast Fourier transformation (FFT) for astigmatism and drift. We selected 315 micrographs from a total of 763 for the reconstruction of DegQ 12-mer, and 796 micrographs from a total of 1835 for the reconstruction of DegQ 24-mer. All the micrographs has the visible Thon rings beyond 1/9 $Å^{-1}$.

All image processing and 3D reconstructions were done using EMAN software (Ludtke et al., 1999, 2001). Particles were selected from the digital micrographs with the boxer program. The contrast transfer functions (CTF) were manually determined with the ctfit program. For 2D analysis, reference-free classification was simply carried out using "refine2d" in EMAN—that incorporates iterative centering, multi-reference alignment, and classification into a single batch script—with an initial class number of 100–200, depending on the data set. The initial models for DegQ 12-mer and 24-mer were generated from reference-free class-averages with the startcsym program. The models were iteratively refined until no further improvement can be obtained. During the reconstruction process, tetrahedral and octahedral symmetries were imposed to 12-mer and 24-mer, respectively. The resolution for the final reconstruction result was determined as 8.2 Å for 12-mer and 6.5 Å for 24-mer based on the 0.5 criterion of Fourier shell correlation (FSC). The final reconstruction models were Gaussian low-pass filtered to the 8.2 Å for 12-mer and 6.5 Å for 24-mer, respectively, with a B factor of 200 $Å^{2}$ imposed.

The rigid body fitting of the crystal structure into the 3D EM map was carried out with the colores program of the Situs software package (Wriggers, 2010; Wriggers et al., 1999). The correlation coefficient between the atom model and EM map was calculated by Situs. The images of the reconstructions and crystal structures were prepared with the University of California, San Francisco Chimera program (Pettersen et al., 2004).

Homology Modeling of DegQ and Flexible Fitting

The homology model of DegQ was generated by Modeler (Sali et al., 1995; Sánchez and Sali, 2000) using the structure of DegP 24-mer (PDB ID: 3CS0) as the template. Subsequently, the atom model of DegQ was obtained by flexible fitting the homology model of DegQ into the EM map of DegQ 24-mer using the MDFF method (Trabuco et al., 2008).

ACCESSION NUMBERS

The cryo-EM density maps of DegQ 12-mer and 24-mer were deposited in Electron Microscopy Data Bank (EMDB) with the access number EMD-5290 and EMD-5289, respectively.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and can be found with this article online at doi:10.1016/j.str.2011.06.013.

ACKNOWLEDGMENTS

We thank Zeng-yi Chang (Peking University) and Hong-Wei Wang (Yale University) for comments; Ning Gao, (Tsinghua University), Qing-tao Shen (Yale University), and Jiansen Jiang (UCLA) for helpful discussion; Yanji Xu for maintenance of cryo-EM. This work was supported by the National Basic Research program of China (2011CB910500/2010CB912400) and the National Natural Science Foundation of China (30830028/30970591). The authors declare no conflict of interest.

Received: March 12, 2011
Revised: June 7, 2011
Accepted: June 20, 2011
Published: September 6, 2011

REFERENCES

Adair, B.D., and Yeager, M. (2007). Electron microscopy of integrins. Methods Enzymol. *426*, 337–373.

Bass, S., Gu, Q., and Christen, A. (1996). Multicopy suppressors of PRC mutant Escherichia coli include two HtrA (DegP) protease homologs (HhoAB), DksA, and a truncated R1pA. J. Bacteriol. *178*, 1154–1161.

Buchner, J., Grallert, H., and Jakob, U. (1998). Analysis of chaperone function using citrate synthase as nonnative substrate protein. Methods Enzymol. *290*, 323–338.

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

Farn, J., and Roberts, M. (2004). Effect of inactivation of the HtrA-like serine protease DegQ on the virulence of Salmonella enterica serovar Typhimurium in mice. Infect. Immun. *72*, 7357–7359.

Jiang, J., Zhang, X., Chen, Y., Wu, Y., Zhou, Z.H., Chang, Z., and Sui, S.F. (2008). Activation of DegP chaperone-protease via formation of large cage-like oligomers upon binding to substrate proteins. Proc. Natl. Acad. Sci. USA *105*, 11939–11944.

Jomaa, A., Damjanovic, D., Leong, V., Ghirlando, R., Iwanczyk, J., and Ortega, J. (2007). The inner cavity of Escherichia coli DegP protein is not essential for molecular chaperone and proteolytic activity. J. Bacteriol. *189*, 706–716.

Kim, D.Y., and Kim, K.K. (2005). Structure and function of HtrA family proteins, the key players in protein quality control. J. Biochem. Mol. Biol. *38*, 266–274.

Kim, D.Y., Kwon, E., Shin, Y.K., Kweon, D.H., and Kim, K.K. (2008). The mechanism of temperature-induced bacterial HtrA activation. J. Mol. Biol. *377*, 410–420.

Kolmar, H., Waller, P.R., and Sauer, R.T. (1996). The DegP and DegQ periplasmic endoproteases of Escherichia coli: specificity for cleavage sites and substrate conformation. J. Bacteriol. *178*, 5925–5929.

Krojer, T., Garrido-Franco, M., Huber, R., Ehrmann, M., and Clausen, T. (2002). Crystal structure of DegP (HtrA) reveals a new protease-chaperone machine. Nature *416*, 455–459.

Krojer, T., Pangerl, K., Kurt, J., Sawa, J., Stingl, C., Mechtler, K., Huber, R., Ehrmann, M., and Clausen, T. (2008a). Interplay of PDZ and protease domain of DegP ensures efficient elimination of misfolded proteins. Proc. Natl. Acad. Sci. USA *105*, 7702–7707.

Krojer, T., Sawa, J., Huber, R., and Clausen, T. (2010). HtrA proteases have a conserved activation mechanism that can be triggered by distinct molecular cues. Nat. Struct. Mol. Biol. *17*, 844–852.

Krojer, T., Sawa, J., Schäfer, E., Saibil, H.R., Ehrmann, M., and Clausen, T. (2008b). Structural basis for the regulated protease and chaperone function of DegP. Nature *453*, 885–890.

Lindquist, S., and Craig, E.A. (1988). The heat-shock proteins. Annu. Rev. Genet. *22*, 631–677.

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

Ludtke, S.J., Baldwin, P.R., and Chiu, W. (1999). EMAN: semiautomated software for high-resolution single-particle reconstructions. J. Struct. Biol. *128*, 82–97.

Ludtke, S.J., Jakana, J., Song, J.L., Chuang, D.T., and Chiu, W. (2001). A 11.5 A single particle reconstruction of GroEL using EMAN. J. Mol. Biol. *314*, 253–262.

Mo, E., Peters, S.E., Willers, C., Maskell, D.J., and Charles, I.G. (2006). Single, double and triple mutants of Salmonella enterica serovar Typhimurium degP (htrA), degQ (hhoA) and degS (hhoB) have diverse phenotypes on exposure to elevated temperature and their growth in vivo is attenuated to different extents. Microb. Pathog. *41*, 174–182.

Onder, O., Turkarslan, S., Sun, D., and Daldal, F. (2008). Overproduction or absence of the periplasmic protease DegP severely compromises bacterial growth in the absence of the dithiol: disulfide oxidoreductase DsbA. Mol. Cell. Proteomics *7*, 875–890.

Pallen, M.J., and Wren, B.W. (1997). The HtrA family of serine proteases. Mol. Microbiol. *26*, 209–221.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. J. Comput. Chem. *25*, 1605–1612.

Sali, A., Potterton, L., Yuan, F., van Vlijmen, H., and Karplus, M. (1995). Evaluation of comparative protein modeling by MODELLER. Proteins *23*, 318–326.

Sánchez, R., and Sali, A. (2000). Comparative protein structure modeling. Introduction and practical examples with modeller. Methods Mol. Biol. *143*, 97–129.

Schweizer, M., Hindennach, I., Garten, W., and Henning, U. (1978). Major proteins of the Escherichia coli outer cell envelope membrane. Interaction of protein II with lipopolysaccharide. Eur. J. Biochem. *82*, 211–217.

Shen, Q.T., Bai, X.C., Chang, L.F., Wu, Y., Wang, H.W., and Sui, S.F. (2009). Bowl-shaped oligomeric structures on membranes as DegP's new functional forms in protein quality control. Proc. Natl. Acad. Sci. USA *106*, 4858–4863.

Sitia, R., and Braakman, I. (2003). Quality control in the endoplasmic reticulum protein factory. Nature *426*, 891–894.

Skorko-Glonek, J., Laskowska, E., Sobiecka-Szkatula, A., and Lipinska, B. (2007). Characterization of the chaperone-like activity of HtrA (DegP) protein from Escherichia coli under the conditions of heat shock. Arch. Biochem. Biophys. *464*, 80–89.

Sobiecka-Szkatula, A., Polit, A., Scire, A., Gieldon, A., Tanfani, F., Szkarlat, Z., Ciarkowski, J., Zurawa-Janicka, D., Skorko-Glonek, J., and Lipinska, B. (2009). Temperature-induced conformational changes within the regulatory loops L1-L2-LA of the HtrA heat-shock protease from Escherichia coli. Biochim. Biophys. Acta *1794*, 1573–1582.

Sohn, J., Grant, R.A., and Sauer, R.T. (2007). Allosteric activation of DegS, a stress sensor PDZ protease. Cell *131*, 572–583.

Sohn, J., Grant, R.A., and Sauer, R.T. (2009). OMP peptides activate the DegS stress-sensor protease by a relief of inhibition mechanism. Structure *17*, 1411–1421.

Spiess, C., Beil, A., and Ehrmann, M. (1999). A temperature-dependent switch from chaperone to protease in a widely conserved heat shock protein. Cell *97*, 339–347.

Strauch, K.L., and Beckwith, J. (1988). An Escherichia coli mutation preventing degradation of abnormal periplasmic proteins. Proc. Natl. Acad. Sci. USA *85*, 1576–1580.

Trabuco, L.G., Villa, E., Mitra, K., Frank, J., and Schulten, K. (2008). Flexible fitting of atomic structures into electron microscopy maps using molecular dynamics. Structure *16*, 673–683.

Waller, P.R., and Sauer, R.T. (1996). Characterization of degQ and degS, Escherichia coli genes encoding homologs of the DegP protease. J. Bacteriol. *178*, 1146–1153.

Walsh, N.P., Alba, B.M., Bose, B., Gross, C.A., and Sauer, R.T. (2003). OMP peptide signals initiate the envelope-stress response by activating DegS protease via relief of inhibition mediated by its PDZ domain. Cell *113*, 61–71.

Wickner, S., Maurizi, M.R., and Gottesman, S. (1999). Posttranslational quality control: folding, refolding, and degrading proteins. Science *286*, 1888–1893.

Wilken, C., Kitzing, K., Kurzbauer, R., Ehrmann, M., and Clausen, T. (2004). Crystal structure of the DegS stress sensor: How a PDZ domain recognizes misfolded protein and activates a protease. Cell *117*, 483–494.

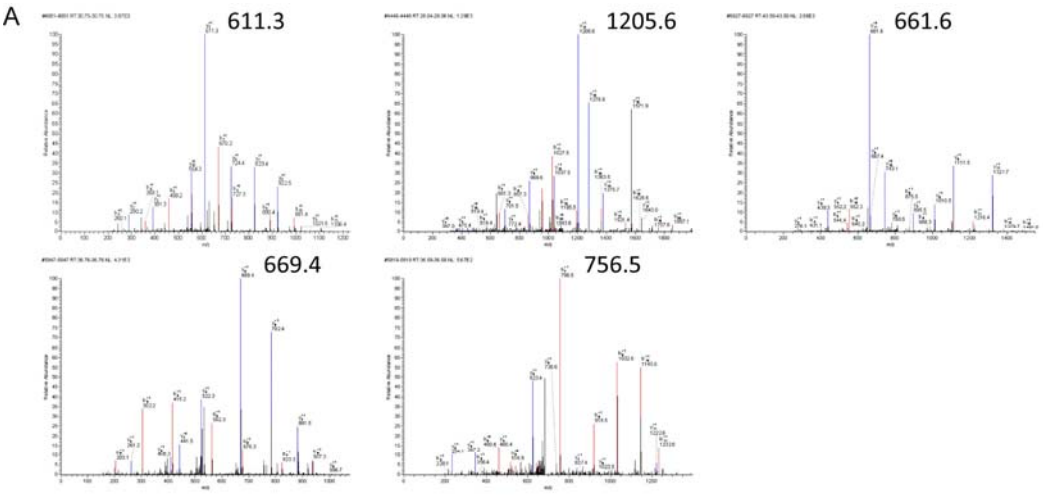
Wriggers, W. (2010). Using Situs for the integration of multi-resolution structures. Biophys. Rev. *2*, 21–27.

Wriggers, W., Milligan, R.A., and McCammon, J.A. (1999). Situs: a package for docking crystal structures into low-resolution maps from electron microscopy. J. Struct. Biol. *125*, 185–195.

Supplemental Information

Characterization of the Structure and Function of *Escherichia coli* DegQ as a Representative of the DegQ-like Proteases of Bacterial HtrA Family Proteins

Xiao-chen Bai, Xi-jiang Pan, Xiao-jing Wang, Yun-ying Ye, Lei-fu Chang, Dong Leng, Jianlin Lei, and Sen-Fang Sui



B

OmpA

MKDLTVLSRWRYSWRILDDNEAQKMKKTAIAIAVALAG
FATVAQAAPKDNTWYTGAKLGWSQYHDTGFINNNGP
THENQLGAGAFGGYQVNPYVGFEMGYDWLGRMPYK
GSVENGAYKAQGVQLTAKLGYPITDDLDVYTRLGGMV
WRADTKSNVYGKNHDTGVSPVFAGGVEYAITPEIATR
LEYQWTNNIGDAHTIGTRPDNGMLSLGVSYRFGQGEA
APVVAPAPAPAPEVQTKHFTLKSDVLFTFNKATLKPEG
QAALDQLYSQLSNLDPKDGSVVVLGYTDRIGSDAYNQ
ALSERRAQSVVDYLISKGIPADKISARGMGESNPVTGN
TCDNVKQRAALIDCLAPDRRVEIEVKGIKDVVTQPQA

Figure S1, related to Figure 1. The peptides mass fingerprinting analysis of the co-purified DegQ substrate.

(A) The MS spectra of the co-purified DegQ substrates. The major peak values of each peptide are marked in larger fonts. (B) The peptides values identified from MS correspond to sub-sequences in OmpA protein (colored in red).

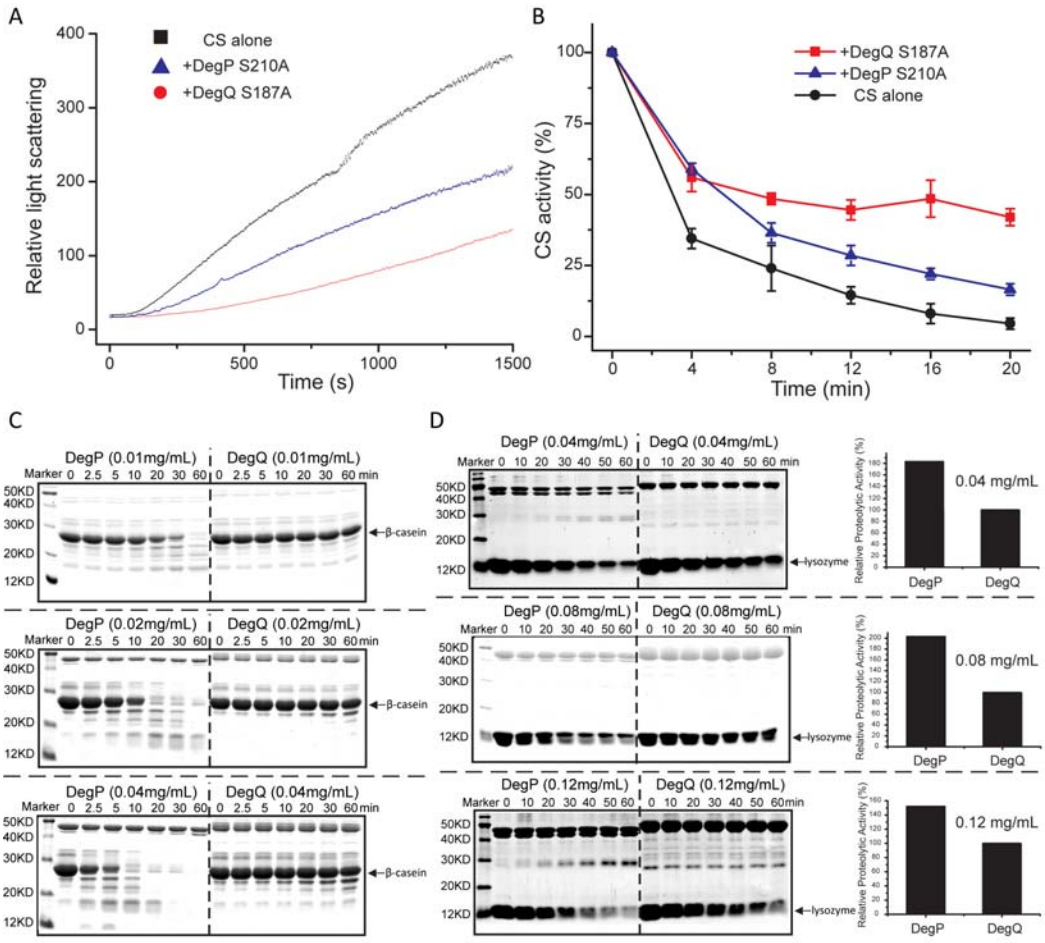


Figure S2, related to Figure 2. Comparison of the chaperone-like and proteolytic activities between DegQ and DegP.

(A) Comparison of the chaperone-like activities between DegQ and DegP in suppressing thermal-induced CS aggregation; (B) Comparison of the leftover CS activities at the indicated time points; (C) The cleavage analysis of DegP and DegQ of three different concentrations against β-casein by SDS-PAGE. The assay was performed at 37°C and stopped at various time points; (D) The cleavage analysis of DegP and DegQ of three different concentrations against DTT denatured lysozyme by SDS-PAGE. The assay was performed at 37°C and stopped at various time points. Quantitation of protein bands of lysozyme was analyzed densitometrically by using *TotalLab* program. Comparison of the relative proteolytic activity of DegP and DegQ against denatured lysozyme was shown on the right, respectively. The relative protease activities were calculated by considering the activity of DegQ as 100%.

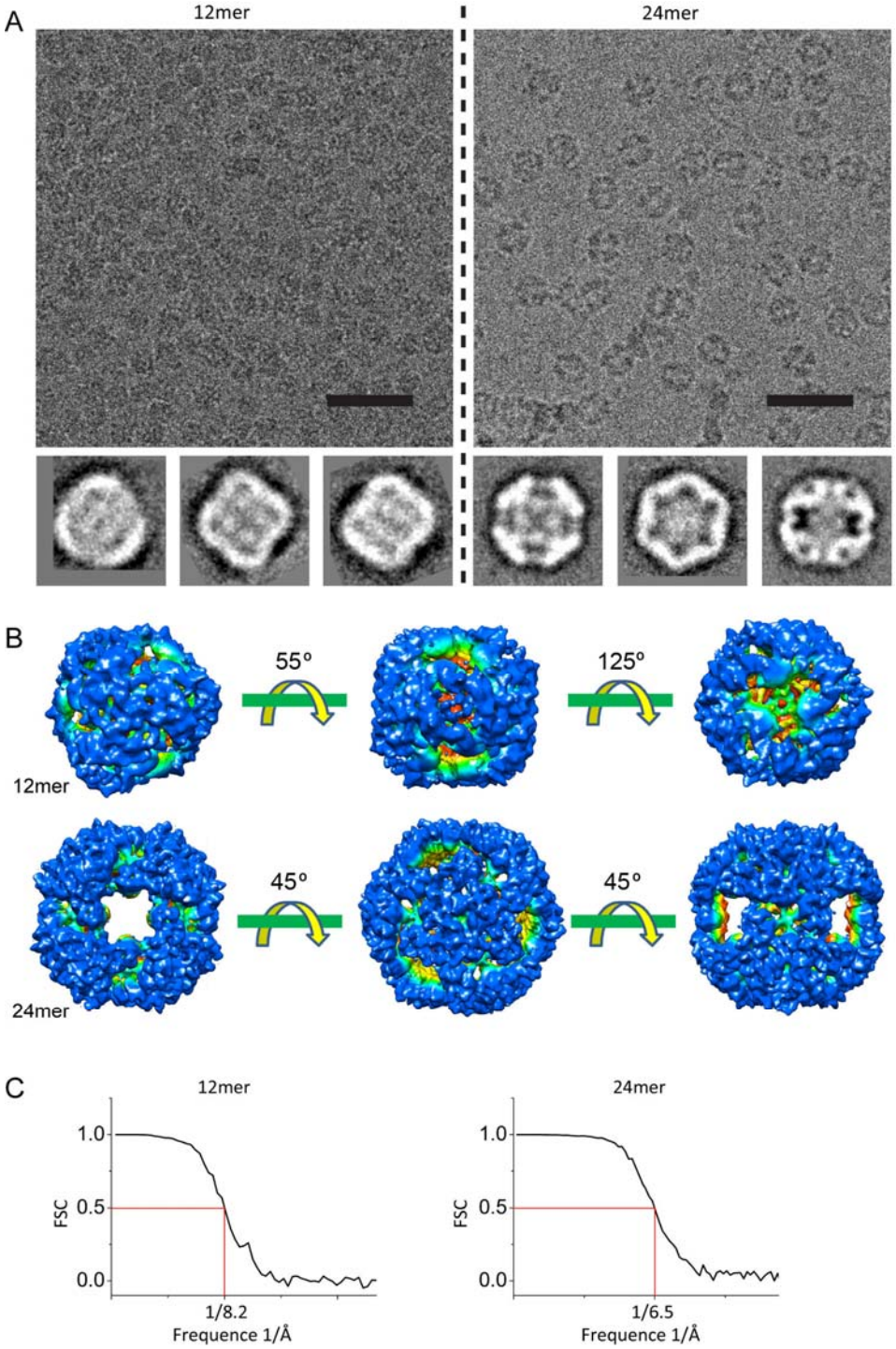


Figure S3, related to Figure 3. Cryo-EM analysis of the DegQ 12-/24-mers.

(A) Representative area of cryo electron micrographs of the DegQ (S187A)/lysozyme complexes 12-mer (left) and DegQ (S187A)/β-casein complexes 24mer (right). Class-averages for each complex are shown below. (B) Surface views of DegQ 12-mer and 24-mer showing their overall architecture. The particle of 12-mer is shown in three different orientations along the three-fold, two-fold and another three-fold axes, respectively. The particle of 24-mer is also shown in three different orientations along the four-fold, three-fold and two-fold axes, respectively. (C) Fourier shell correlation of DegQ 12- and 24-mers. A threshold of 0.5 was used to evaluate the resolution of DegQ 12- (left) and DegQ 24-mer (right).

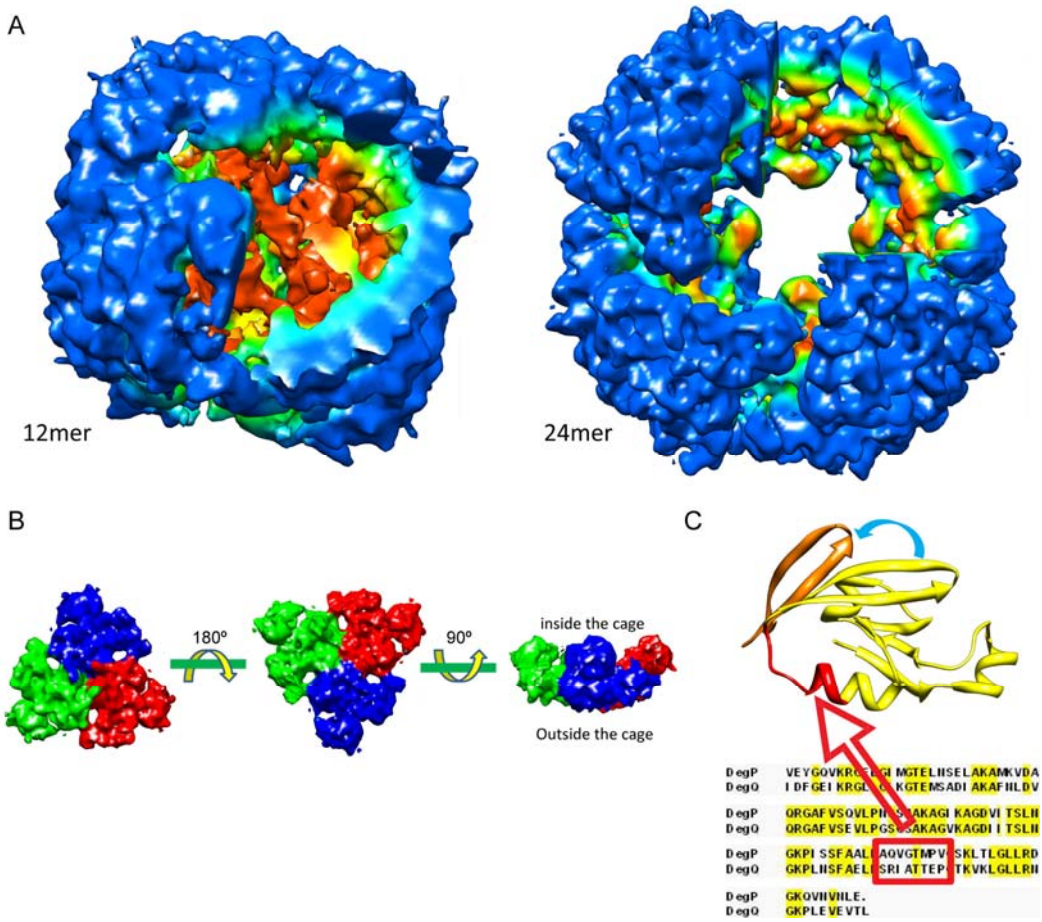


Figure S4, related to Figure 4. The cryo-EM structures of DegQ 12-/24-mer.

(A) A proportion of the volumes were removed from DegQ 12- (left) and 24-mer (right). The addition densities (colored in red) were detected in the center of the DegQ 12-mer. (B) A trimer extracted from DegQ 24-mer shown in three different orientations. Each monomer from one trimer is colored differently. (C) The non-conserved linker connected to β19/20. Sequence alignment showed that the linker connected to β19/20 is not conserved between DegP and DegQ (indicated by the red box), and thus the manual adjustment of β19/20 is feasible (indicated by the blue arrow).