Bowl-shaped oligomeric structures on membranes as DegP's new functional forms in protein quality control

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In the periplasm of *Escherichia coli*, DegP (also known as HtrA), which has both chaperone-like and proteolytic activities, prevents the accumulation of toxic misfolded and unfolded polypeptides. In solution, upon binding to denatured proteins, DegP forms large cage-like structures. Here, we show that DegP forms a range of bowl-shaped structures, independent of substrate proteins, each with a 4-, 5-, or 6-fold symmetry and all with a DegP trimer as the structural unit, on lipid membranes. These membrane-bound DegP assemblies have the capacity to recruit and process substrates in the bowl chamber, and they exhibit higher proteolytic and lower chaperone-like activities than DegP in solution. Our findings imply that DegP might regulate its dual roles during protein quality control, depending on its assembly state in the narrow bacterial envelope.

chaperone | protease | HtrA | cryo-electron microscopy

Proteins damaged by heat shock or other stresses are serious hazards for cells, especially if they accumulate into large aggregates. Cells have developed a sophisticated quality-control system involving molecular chaperones and proteases to reduce the amount of misfolded or unfolded proteins (1). DegP from *Escherichia coli* (also known as HtrA in mammalian cells), exhibiting both refolding and proteolytic activity, plays a central role in protein quality control in the bacterial envelope (2).

DegP is essential for the growth of *E. coli* at high temperatures (37–45 °C), because it eliminates unfolded and damaged proteins efficiently by proteolysis (3). At low temperature (25 °C), DegP mediates the encapsulation and/or refolding of misfolded proteins via its chaperone-like activity to prevent aggregation (2, 4). Biochemical assays and crystallographic analyses have indicated that purified DegP exists mainly as a hexamer with 2 homotrimers stacked in a face-to-face manner (5, 6). This hexameric model, however, does not explain how DegP's proteolytic and chaperone-like activities are regulated. Recent studies from Krojer et al. (7) and our group (8) have shown that hexameric DegP assembles into large, catalytically-active spherical structures around its substrates. These spheres are 12- or 24-meric multimers composed of 4 or 8 homotrimers, respectively, depending on the size of the substrate. This transformation extracts loop LA in DegP from the active site of the neighboring molecule in the next trimer and releases loops L1 and L2 to establish a functional proteolytic site to degrade chromogenic (7, 9) or commonly used substrates (8). These multimers also exhibit chaperone-like activity by encapsulating the folded promoters of outer membrane proteins (7) and preventing the aggregation of denatured lysozyme in solution (8). The discovery of these enormous spherical multimers in solution has greatly enhanced our knowledge of the molecular mechanism of DegP activity in the *E. coli* periplasm.

DegP is involved in the quality control of several important membrane-related proteins in the narrow *E. coli* envelope (10); therefore, its relationship with the cell membrane is of great interest. Indeed, some studies have suggested that DegP has a high affinity for the periplasmic side of the inner membrane (3, 11). In vivo, the overproduction of DegP S210A leads to the localization of such outer membrane proteins as OmpF and OmpC on the inner membrane (12, 13). In vitro, DegP interacts with liposomes containing phosphatidylglycerol (PG) phospholipids or cardiolipin (CL) but not with those containing only phosphatidylethanolamine (PE) phospholipids, indicating the existence of electrostatic interactions among DegP's basic residues and the negatively-charged headgroups of PG and CL (11, 14). In the PG/DegP protein–liposome system, membrane binding leads to slight changes in the secondary structure of DegP and a remarkable decrease in its thermal stability, suggesting a change in its tertiary structure (11). DegP's proteolytic activity also increases in the presence of liposomes at temperatures between 37 °C and 45 °C (11).

To further explore the structure and function of DegP, we examined its structure on lipid membranes via electron microscopy (EM) and single-particle analysis, and we performed proteolytic and chaperone activity assays using various types of membranes.

Results

Lipid Membranes Induce DegP Oligomerization. We first examined the binding of WT DegP with lipid vesicles made of *E. coli* total lipid by using cosedimentation assays. Significant liposomal binding was observed as the lipid/protein ratio increased (Fig. S1*A*). At mass ratios >9:1, >70% of the DegP cosediments were bound to liposomes (i.e., near saturation). The binding reaction was fast, reaching half maximum within 10 min [surface plasmon resonance (SPR); see below]. The proteolytic activity-null mutant S210A behaved identically to the WT protein in liposome binding assays. We separated the DegP–liposome complex from free DegP by gel filtration after >1 h of incubation at 37 °C and examined the protein–liposome complex by negative-staining EM (Fig. 1*A*). As expected, populated protein particles appeared on the liposome surface; however, the particles were larger than expected (i.e., they were larger than when in solution in the absence of substrate).

To further analyze the DegP assemblies on the membrane, we used the lipid monolayer technique to obtain higher-quality images. After incubation under a monolayer made of *E. coli* total lipid at 37 °C for 6 h, the DegP/membrane was removed with a

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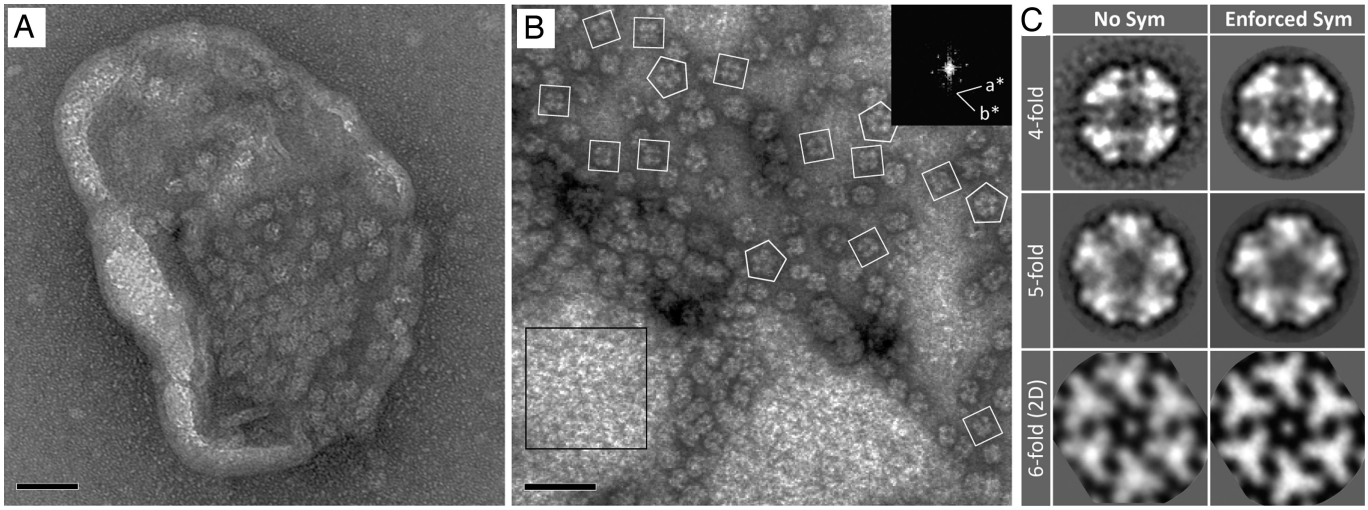

Fig. 1. Visualization of DegP oligomeric structures on membranes by using EM. (*A*) Negatively-stained DegP on the surface of liposomes composed of *E. coli* total lipid. (*B*) Negatively-stained DegP on lipid monolayers made of *E. coli* total lipid. Particles with 4 obvious lobes are marked with squares, and those with 5 lobes are marked with pentagons. Patches of 2D crystals of DegP are located in the lower part of the micrograph; a selected area of the crystal is shown together with its Fourier transform (*Inset*). The scale bars in *A* and *B* correspond to 50 nm. (*C*) Class averages for the oligomeric structures with 4, 5, and 6 (projection map calculated from the 2D crystal) units with or without respective rotational symmetry. The box length in each panel is 25 nm.

carbon-coated copper grid, washed intensively, negatively stained, and examined by EM. Large oligomeric particles similar to those observed on the liposomes were found on the lipid monolayers (Fig. 1*B*). The particles exhibited a strong preferential orientation on the grids, and most appeared to have 4 or 5 protein densities surrounding a heavily-stained pore at the center. 2D alignment and classification of the particles indicated 2 prominent populations with 4- and 5-fold symmetries, respectively (Fig. 1*C*). Approximately 90% of the particles were 19 nm in diameter with 4-fold symmetry, whereas the other 10% were 23 nm in diameter with 5-fold symmetry. Class-average analysis clearly showed that the asymmetric units in the 2 types of symmetrical particles had very similar triangular shapes. We also occasionally observed particles with 6-fold symmetry that tended to form small patches of 2D crystals with a hexagonal lattice (Fig. 1*B*). A projection map calculated from the 2D crystals illustrated a 6-fold ring-like structure consisting of 6 triangular densities (Fig. 1*C*).

Purified WT and S210A DegP exist mainly as hexamers (dimers of trimers) in solution (5, 6), and the binding of denatured protein substrates transforms the hexamers into larger 12- and 24-meric cages (7, 8). Our observation indicates that membrane-bound DegP assembles independently of the substrate. To exclude the possibility that the autocleaved products of WT DegP might be present on the assembly, we repeated all of the above experiments with the proteolytic activity-null mutant DegP S210A; no differences compared with WT DegP were detected. However, no similar oligomeric structures containing DegP were observed in bulk under the membrane or in solution. The role of the membrane in DegP assembly in the absence of substrate suggests an alternative mechanism for the assembly of DegP in the cell envelope and a different biological role than that described for DegP in solution.

DegP Forms Bowl-Shaped Structures on Membranes. We next solved 3D reconstructions of the membrane-induced DegP assemblies to identify any similarities/differences with the substrate-induced assemblies seen in solution. Using the random-conical tilt (RCT) method (15), we obtained 3D density maps of 4- and 5-fold assemblies from negatively-stained particles on membranes (Fig. S2). The maps revealed bowl-shaped structures with their symmetrical axis perpendicular to the membrane plane. Using these reconstructions as initial models, we refined the structures via projection-matching refinement (16) on untilted and tilted micrographs of the vitrified specimen. Thus, we ultimately obtained 3D reconstructions of the 4- and 5- fold bowl assemblies at resolutions of ≈18 and 26 Å, respectively (Fig. 2*A*, Figs. S3 and S4). Both types of bowls consist of very similar building blocks with pseudo 3-fold symmetry, with the shape and size characteristics of a DegP trimer. Four trimers associate via lateral interactions forming the 4-fold bowl with a diameter of 18 nm and a height of 9 nm, whereas five trimers assemble in the same way, forming a 5-fold bowl with a diameter of 23 nm and a height of 7 nm.

The 4-fold bowl assembly contains 12 monomers in the shape of a pyramid, exactly one-half of the 24-mer structure induced by the substrate in solution (7, 8). Agreeing with this result, the half 12-mer from the crystal structure of the 24-mer could be docked fairly well into the EM density map (Fig. 2*B*). The only major discrepancies occur at the intertrimer interfaces, where the PDZ1–PDZ2* interaction appear to be weaker or more flexible than in the crystal structure, and at the edges of the bowls, where the PDZ2 domain is quite flexible (at the lower threshold the bowl edge is less well defined). It has been shown that the PDZ1 and PDZ2 domains on the outer surface of the 24-mer are responsible for its interaction with membranes (7), and this finding implies that the bowl assembly interacts with membranes via its bottom rather than its edge. This idea was confirmed by our RCT reconstruction, which was prepared with special attention to the orientation of the lipid monolayer during membrane transfer, EM, and image processing. Following the same PDZ1–PDZ2* interaction strategy, 5 trimers from the 24-mer atomic model could be fitted into the 5-fold bowl assembly with a larger open chamber of the bowl by tilting them further toward the membrane surface (Fig. 2*C* and Fig. S4*B*). In the same way in which the trimers were assembled, we were able to dock 6 copies of the trimeric atomic models onto the 2D projection map of the hexagonal 2D crystals of DegP on the lipid monolayer, where the 6 trimers formed a planar platform (Fig. 2*C*). These observations indicate that the trimers can assemble into oligomers with various curvatures through relatively flexible interactions between the PDZ1 and PDZ2* domains or a flexible linker between the protease and PDZ1 domains (Figs. S4*C* and S5). The membrane

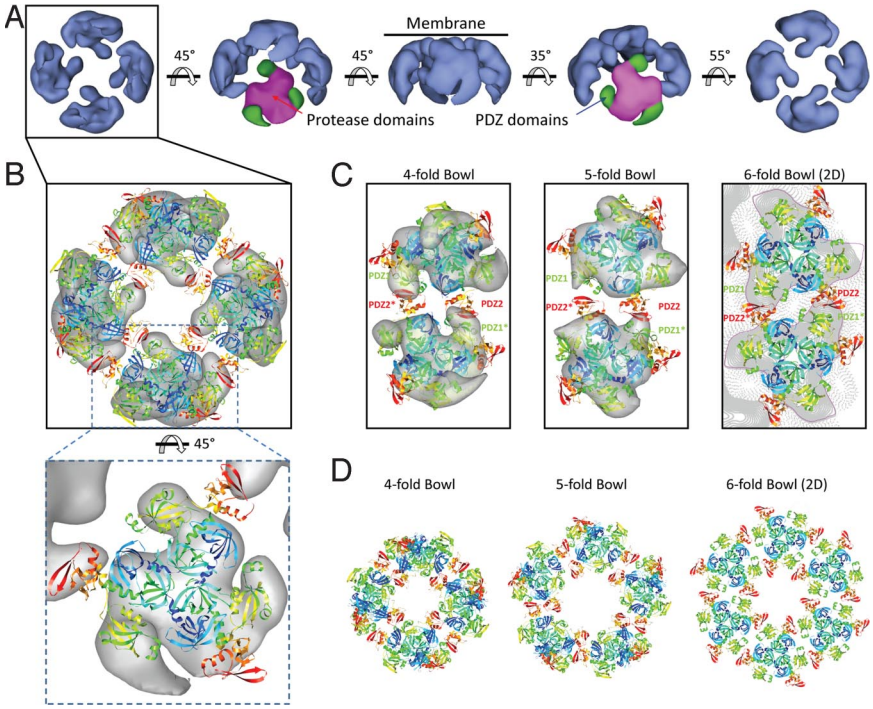

Fig. 2. Reconstruction of bowl-shaped DegP assemblies on the membrane. (*A*) 3D reconstruction of bowl-shaped DegP assemblies with 4-fold symmetry on the membrane. The membrane is at the back of the image in the first view and at the front in the fifth view. Its location in the third view is indicated by a line. The protease domains are pink, and the PDZ domains are green in the second and fourth views. (*B*) Half of the atomic model of the DegP 24-mer (Protein Data Bank ID code 3CS0) docked on the 4-fold bowl-shaped map. A close-up of 1 trimer at its 3-fold axis view is also shown. The PDZ1, PDZ2, and protease domains are green, red, and blue, respectively. This color coding also applies to *C* and *D*. (*C*) Comparison of the trimer interfaces of the 3 bowl-shaped assemblies to the atomic models of trimers docked in the maps. All 3 are top views of the bowls with the membrane on the far side. (*D*) Pseudo atomic models of the 4-, 5-, and 6-fold bowl-shaped assemblies of DegP on membranes. The views are the same as those described in *C*.

apparently provides a platform for the formation of these various assemblies (Fig. 2*D*).

Membrane Lipid Composition Affects the Bowl-Shaped Assembly of DegP. Unlike the soluble 12- and 24-meric multimers formed in the presence of denatured proteins, the formation of the bowl-shaped assemblies depends on the surface charge and fluidity of the associated membrane.

The binding of DegP to the membrane is the first step in the formation of the assembly. DegP has a higher affinity for liposomes containing negatively-charged lipids (11). The crystal structure of the DegP trimer revealed that the PDZ domains, especially their back sides, are strongly positively charged, whereas the protease domains are slightly negatively charged. These PDZ domains have been reported to take charge of the binding of DegP to the negatively-charged headgroups in phospholipids (7). This notion was confirmed by our binding assays of the protein on membranes composed of different lipids. DegP has a high affinity for liposomes made of negatively-charged lipids such as 1,2-dioleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (DOPG) or 1,2-dipalmitoyl-sn-glycero-3-[phospho-rac-(3-lysyl (1-glycerol))] (DPPG), whereas it has weak affinity for liposomes made of neutral lipids such as 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine (DOPE) (Fig. S1*B*). The same bowl-shaped assemblies were observed by EM on DOPG monolayers incubated with DegP as those on *E. coli* total lipid monolayers; however, few proteins were available on the DOPE monolayers (Fig. 3*A*). Membrane pressure measurements of monolayers with various compositions indicated that DegP does not affect membrane pressure. This finding suggests that no portion of DegP is inserted into the membrane, and thus the major driving force for DegP's binding to the membrane is ionic interactions between the positively-charged PDZ domains and the negatively-charged phospholipid headgroups.

After DegP binds to negatively-charged membranes, the reorganization of DegP is another vital step in the formation of new oligomers. Membrane fluidity, which depends on the extent of lipid saturation, has a strong influence on this process. We examined the assembly of DegP on membranes containing DOPG/DOPE and DPPG/1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE), which have similar surface charges but different phase-transition temperatures, at 16 °C and 60 °C, respectively. The lateral diffusion of DegP on these 2 types of membranes is significantly different at 37 °C. The binding of DegP to the DOPG/DOPE and DPPG/DPPE liposomes both reached the same saturation level within 30 min at 37 °C (SPR results below). However, when examined under EM, the 2 membrane systems showed distinct properties. On one hand, 10-min incubation was sufficient for the formation of bowl-shaped structures on DOPG/DOPE monolayers and >30-min incubation could induce a large percentage of the bowl-shaped assemblies. However, only small particles resembling DegP trimers or hexamers were observed on the DPPG/DPPE monolayers within a similar period of incubation (Fig. 3*C*). When the incubation time was prolonged to 240 min, large oligomers were observed on both the DOPG/DOPE and DPPG/DPPE membranes (Fig. 3*C*). Monitoring the assembly process indicated that the assembly of large oligomers took longer to form on the DPPG/DPPE membranes than on the DOPG/DOPE membranes (Fig. 3*D*). These data suggest that after DegP binds to a membrane the membrane's fluidity helps individual molecules of DegP gather and assemble into bowl-shaped oligomers.

Bowl-Shaped DegP Assemblies on Membranes Have Higher Proteolytic Activity but Lower Chaperone-Like Activity. As an important quality-control factor, DegP possesses both proteolytic and chaper-

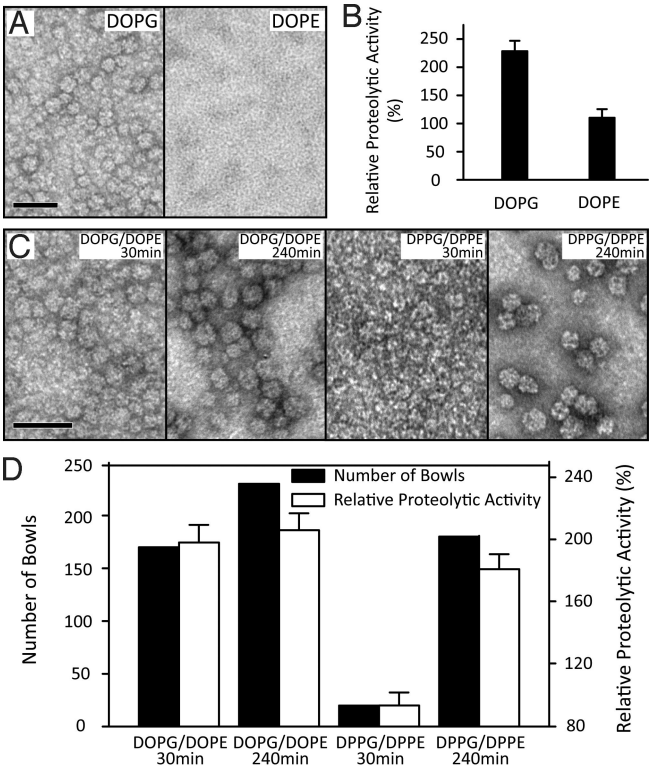

Fig. 3. Membrane lipid composition affects the formation of bowl-shaped DegP assemblies. (*A*) EM images of DegP under monolayers with different charges. The scale bar represents 50 nm. (*B*) The relative proteolytic activities of DegP against β-casein in the presence of DOPG and DOPE vesicles compared with that of DegP in solution. (*C*) EM images of DegP under monolayers with different fluidities at different incubation times. The molar ratio of PG to PE in all membranes is 1:4. The scale bar represents 50 nm. (*D*) The number of bowl-shaped assemblies within a specific area (0.8 μm^2) from representative EM images of the different membrane conditions in *C* and the respective relative proteolytic activity of DegP.

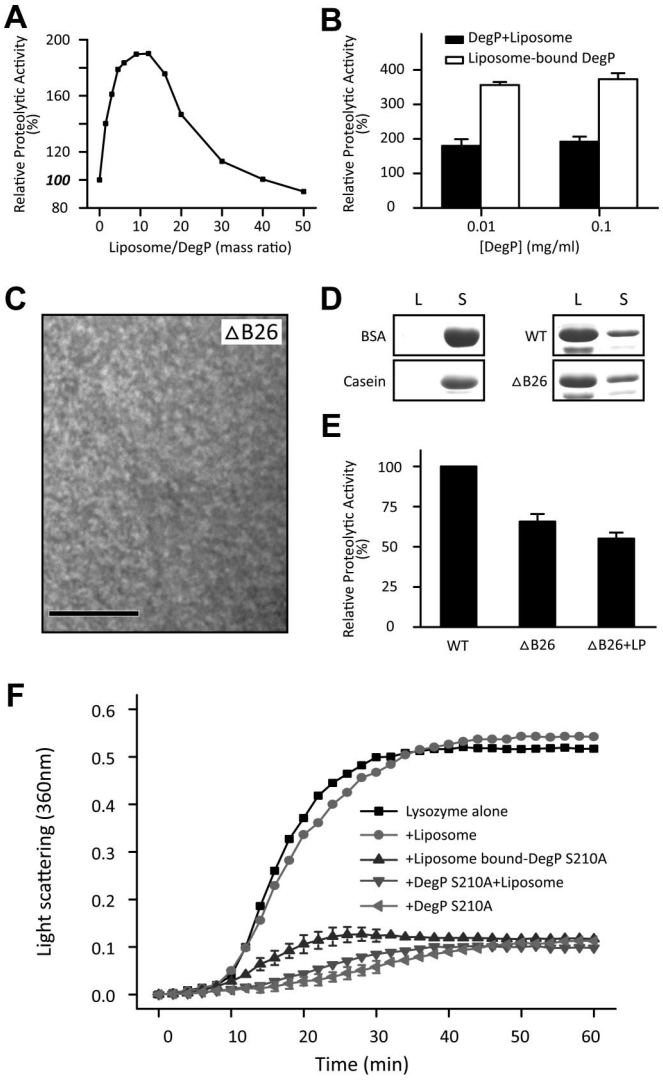

Fig. 4. Bowl-shaped DegP assemblies on membranes have higher proteolytic activity but lower chaperone-like activity than DegP in solution. (*A*) The relative proteolytic activity of WT DegP at different lipid/protein mass ratios. A fixed concentration of 0.1 mg/mL WT DegP was incubated with various amounts of DOPG/DOPE liposomes (molar ratio, 1:4) for 2 h before adding 2 mg/mL β-casein for the proteolysis assay. The proteolytic activity of WT DegP in solution was used as a control. (*B*) Comparison of the proteolytic activity in the DegP–liposome mixture to that of separated liposome-bound DegP. The assay was conducted as in *A* with a fixed lipid/mass ratio of 9:1. Two concentrations of DegP were used; for each, the activity of the protein alone in solution was used as a control. (*C*) EM image of the DegP mutant ΔB26 on an *E. coli* total lipid monolayer. The scale bar represents 50 nm. (*D*) Cosedimentation assay of ΔB26 on an *E. coli* total lipid vesicle using BSA, β-casein, and WT DegP as controls. (*E*) Comparison of the proteolytic activities of WT in solution, ΔB26 in solution, and ΔB26 with liposomes. The experimental setup was as in *A*; the concentration of DegP was the same (0.1 mg/mL) as in the assays using WT in solution as the control. (*F*) The chaperone-like activity of DegP S210A protected DTT-denatured lysozyme against aggregation. The light-scattering values for the incubated samples were recorded at 360 nm over time.

one-like activities. Thus, we examined these factors in the presence of membranes.

DegP exhibits stronger proteolytic activity toward naturally-unfolded β-casein in the presence of the membrane (11). We systematically analyzed the proteolytic activity of DegP by using 4 common substrates, β-casein, denatured malate dehydrogenase (MDH), lysozyme, and α-lactalbumin (α-LA), in the presence and absence of liposomes. At 42 °C, the proteolytic activity of DegP toward all 4 substrates increased with increasing ratio of liposome to DegP (Fig. 4*A* and Fig. S6*A*). We next separated the liposome-bound and unbound DegP molecules via gel filtration. After separation from soluble DegP molecules via gel filtration, liposome-bound DegP degraded β-casein more quickly and exhibited greater proteolytic activity (Fig. 4*B*). This finding is in agreement with our binding assay results, which indicated that more DegP was bound to the liposome fraction at a higher lipid-to-protein ratio. Interestingly, the proteolytic activity of the protein continued to increase as the lipid/protein ratio increased. It peaked at ≈12:1 then decayed as more liposomes were added and fully lowered to the level of DegP in solution at a lipid-to-protein ratio of 50:1 (Fig. 4*A*). This result suggests that there is a critical concentration of membrane-bound DegP at which proteolytic activity peaks and led us to hypothesize that the increased proteolytic activity is attributable to the bowl-shaped assemblies. Supporting this hypothesis, our results showed that the proteolytic activity of DegP was influenced by the charge and fluidity of the membrane, similar to assembly formation. The DOPG liposomes significantly increased the proteolytic activity of DegP, whereas the DOPE liposomes had little effect (Fig. 3*B*). In another experiment, the incubation for 30 min with DOPG/DOPE liposomes brought about a 2-fold increase in DegP proteolytic activity; the same period of incubation with DPPG/DPPE liposomes did not induce a significant change in activity (Fig. 3*D*). When the incubation time was extended to 240 min, DegP proteolytic activity in-

creased to the same level on both types of membranes (Fig. 3*D*). These results support the notion that DegP bowl-shaped assemblies are responsible for the increase in proteolytic activity.

More direct evidence was obtained from mutation experiments based on structural analyses. Previous data (7, 8) and the electron microscopic reconstruction conducted in this study demonstrated that interplay between the PDZ1 and PDZ2* domains is involved in the formation of bowl-shaped assemblies on the membrane. We previously showed that DegP ΔB26 was impaired in its ability to form assemblies (8). In the present study, the membrane binding ability of DegP ΔB26 was similar to that of WT; however, it was unable to form larger oligomers (Fig. 4 *C* and *D*). In agreement with our hypothesis, incubating DegP ΔB26 with liposomes did not increase its proteolytic activity; instead, it showed a slightly lower level of proteolytic activity compared with DegP ΔB26 in solution (Fig. 4*E*). Therefore, preventing DegP assembly formation hampers the protein's proteolytic activity on membranes. These observations strongly suggest that membrane-induced bowl-shaped assemblies of DegP have greater proteolytic activity than trimers or hexamers before assembly. We also examined the proteolytic activity of DegP toward natural proteins such as BSA but did not observe any proteolysis. This finding indicates that only misfolded or partially misfolded proteins are recognized by membrane-bound DegP.

As a chaperone, DegP could encapsulate misfolded proteins such as DTT-denatured lysozyme from aggregates in solution (4, 8). We thus examined DegP's chaperone-like activity in the presence of liposomes. The chaperone-like activity (i.e., prevention of lysozyme aggregation) in a mixture of membrane-bound and unbound DegP S210A was similar to that of DegP S210A in solution (Fig. 4*F*). When membrane-bound DegP S210A was separated from the unbound fraction and tested for its chaperone-like activity, partially-reduced activity was observed compared with an equal amount of DegP in solution during the early stages of the reaction (Fig. 4*F*). After 40 min of incubation, the chaperone-like activity in the membrane fraction recovered, probably because of the establishment of a new equilibrium between membrane-bound and -unbound 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, membrane-bound DegP S210A had significantly less protecting effect against the thermal-induced aggregation of CS than DegP S210A in solution (Fig. S7*A*). Further enzymatic activity assay of CS in the 2 systems proved that membrane-bound DegP S210A has lower protection against the denatured protein than DegP S210A in solution (Fig. S7*B*).

DegP Bowl-Shaped Assemblies Recruit and Process Substrates in the Bowl Chamber. The docking of the crystal structure in our EM reconstruction map revealed that the substrate-binding and proteolytic activity sites are located on the inner surface of the bowl, implying that the substrates can be recruited directly to the center of the bowl on the membrane. We monitored the substrate recruitment process on lipid membranes by SPR (see *Materials and Methods*). As in our cosedimentation assays, we observed the binding of DegP S210A to the negatively-charged membrane. When β-casein was added to the system, significant adsorption of the substrate on the membrane surface was detected in the S210A–membrane system (Fig. 5*A*). In contrast, when DegP was absent, no β-casein binding was detected.

To further illustrate the location of the substrates in the membrane-bound DegP assemblies, we examined a β-casein/S210A/membrane specimen by EM. In contrast to the DegP/membrane specimen, where a heavily-stained hole was present in the middle of the bowl, an additional protein density occupying the bowl's center was observed when β-casein was available in the bulk solution (Fig. 5*B*). To elucidate the preferential

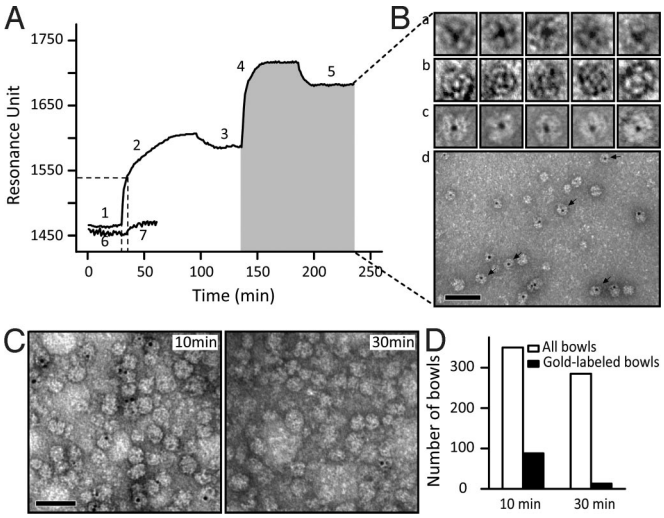

Fig. 5. Bowl-shaped DegP assemblies recruit and process substrates in the bowl chamber. (*A*) Real-time SPR curve of S210A DegP binding to an *E. coli* total lipid monolayer and the further adsorption of β-casein (lines 1–5) shown with the control (adsorption of β-casein alone; lines 6 and 7). Lines 1 and 6, PBS washing after coating the slide with BSA; line 2, adsorption of DegP to the membrane; line 3, PBS washing; line 4, adsorption of β-casein to the DegP–membrane complex; line 5, PBS washing; line 7, adsorption of β-casein directly to the membrane. The time required for half-maximal binding of DegP to the membrane is indicated by dashed lines. (*B*) Electron micrographs showing the bowl-shaped DegP assemblies with bound substrates. (*a*) Representative single particles of DegP on the membranes. (*b*) The particles with bound β-casein, (*c*) Representative DegP bowls with gold-labeled β-casein located in the middle each bowl. (*d*) A larger view showing that >30% of the DegP bowls had gold-labeled β-casein at the center. The box size in *a*–*c* rows is 25 nm; the scale bar in *d* represents 50 nm. (*C*) Representative micrographs of WT DegP bowls on membranes incubated with gold-labeled β-casein at 2 different time points. The scale bar represents 50 nm. (*D*) The number of gold-labeled bowls of WT DegP in 0.8-μm^2 areas from representative micrographs at 2 different time points.

affinity of substrates for membrane-bound DegP, we generated colloidal gold-labeled β-casein and incubated the substrate with the DegP/membrane. A number of gold particles were seen to accumulate in the middle of the bowl-shaped assemblies on the DegP S210A-decorated monolayers within 15 min of incubation (Fig. 5*B*). The number of gold-labeled bowls did not change over time because S210A does not digest substrate. Notably, a similar accumulation of gold was detected on the WT-decorated monolayers at the start of incubation; however, the number of gold-labeled bowls decreased significantly as the incubation period progressed >30 min (Fig. 5 *C* and *D*). This indicates that the bowl-shaped assemblies are able to both recruit substrates specifically to their activity chamber and to degrade the substrates.

Discussion

Since the discovery of DegP's dual role as a protease and chaperone in protein quality control, much progress has been made toward understanding the relevant molecular mechanisms. The atomic model of DegP as hexamers enhanced our knowledge of the protein's structure in its inactive state (5). The recent discovery that DegP encapsulates its substrates in solution by forming spherical assemblies provided a snapshot of the protein in its active form (7, 8). These data, however, did not indicate how DegP switches between protease and chaperone roles via formation of large cage-like structures, especially in the narrow periplasm of the bacterial envelope.

The present study revealed that DegP, independent of the substrates, forms bowl-shaped assemblies on lipid membranes.

Similar to the spherical structures, the bowl-shaped assemblies also show proteolytic and chaperone-like activities, indicating that the bowls are another form of active DegP and that they may play vital physiological roles in vivo. As suggested by Krojer et al. (7), spherical DegP may serve as a chaperone pore between the inner and outer membranes in bacteria and help correct the folding and localization of membrane proteins in the outer membrane. Our current observation that the membrane-bound bowl-shaped assemblies of DegP have dramatically higher proteolytic and lower chaperone-like activities than unbound DegP in solution indicates that the bowl-shaped DegP may serve mainly as a protease to degrade unfolded proteins before it grows into a sphere. Despite the various rotational symmetries (4-, 5-, or 6-fold), all of the bowl-shaped assemblies consist of DegP trimers, which are also the building blocks of 12- and 24-meric spherical assemblies in solution. The trimers in the bowls and spheres interact with each other in a similar way. Thus, it is plausible that the bowl-shaped assemblies recruit new trimers or hexamers and form intact cage-like structures after substrate recruitment. Our current results implied an appealing model that the assembly state of DegP is responsible for the switch between its 2 roles. Further studies are necessary to clarify this hypothesis. The recent work by Krojer et al. (7) and our group (8) has proven the presence of the cage-like DegP assemblies in vivo. The bowl-shaped assemblies observed in this work are likely to be the structural intermediates of the active cage-like assemblies in the periplasm. Direct evidence of their existence in vivo should be sought in future studies with various techniques, i.e., EM tomography.

DegP lies downstream of the CPX (17) and δ^E (18) signal transduction pathways as a responder to heat shock and other stresses. DegP is up-regulated under these conditions to remove misfolded or unfolded proteins as quickly as possible and to protect cells from abnormal aggregation or even death (3). The bowl's open proteolytic chamber with various curvatures lends the convenient access to various substrates thus may help increase the proteolytic efficiency and rescue cells from sudden shocks. It appears that the ratio of bowl- to sphere-like assemblies of DegP is regulated by the properties of the membrane, which depend on temperature and lipid composition; substrate concentration, which is affected by stress; and DegP concentration, which is regulated by signal transduction. Exactly how the transition between DegP's different oligomeric states is regulated requires further elaboration.

Materials and Methods

Full details are available in *SI Text*.

EM Observation of DegP on Membrane. EM observation of DegP on liposomes or monolayers was carried out as described (19).

3D Reconstruction. The 3D reconstruction was calculated by using a combination of the RCT technique and projection-match refinement (15). Atomic trimers derived from DegP 24-mer in solution were used to fit the EM 3D map in UCSF Chimera (20) based on local optimization strategy.

Proteolytic and Chaperone-Like Activities Assays. The proteolytic activity of DegP or DegP/liposome against β-casein, MDH, egg white lysozyme, and α-LA was performed as described (6, 21). The chaperone-like activities of DegP or DegP/liposome against DTT-denatured lysozyme or thermal-denatured CS were measured as described (4, 8, 22).

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