Structural analysis of DegS, a stress sensor of the bacterial periplasm

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Abstract Regulated proteolysis is a key event in transmembrane signalling between intracellular compartments. In *Escherichia coli* the membrane-bound protease DegS has been identified as the periplasmic stress sensor for unfolded outer membrane proteins (OMPs). DegS inititates a proteolytic cascade resulting in the release of σ^E the transcription factor of periplasmic genes. The crystal structure of DegS protease reported at 2.2 Å resolution reveals a trimeric complex with the monomeric protease domain in an inhibited state followed by the inhibitory PDZ domain. Noteably, domain architecture and communication of DegS are remarkably to homologous proteins known to date. Here the domain interface is mechanically locked by three intradomain salt bridges. Co-crystallisation trials in the presence of a 10-residue activating peptide did not result in significant structural intradomain shifts nor distortions in the crystal packing. These observations imply a mode of activation indicative of peptide-induced structural shifts imposed to the protease domain rather than disturbing the PDZ–protease interface.

Keywords: X-ray; Molecular replacement; Htra; Outer membrane protein; Protease; PDZ domain

1. Introduction

Signalling events between cellular compartments are often accomplished by regulated proteolysis of membrane proteins after activation of transmembrane proteases. Potential substrates of these proteases include integral transcription factors or anti-sigma factors; the mechanism of activation displayed appears to be conserved from bacterial to human cells. One of the best studied examples occurs at the endoplasmic reticulum membrane where two transcription factors, ATF6 and SREBP, are cleaved by integral membrane proteases, first within their luminal and successively in the transmembrane part [1]. Accordingly the release of the soluble domains and their translocation to the nucleus triggers the expression of proteins destined for the unfolded protein response and cholesterol biosynthesis [2,3].

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Abbreviations: OMP, outer membrane protein

The periplasmic stress response in *Escherichia coli* is tightly controlled by the σ^E-dependent pathway which controls the expression of many proteins most importantly chaperones, proteases and enzymes involved in lipid A synthesis [4]. While the stress signal is initially sensed in the periplasmic space a signal cascade has to be directed across the inner membrane to yield amplification in the cytosolic compartment. Three membrane-bound enzymes RseA, DegS and YaeL are key players in this signal pathway. The stress response may be induced by misfolded outer membrane proteins (OMPs) due to environmental stress followed by the stepwise degradation of the inner membrane protein RseA, an anti-σ^E-factor [5,6].

The proteolytic cascade is initiated by the periplasmic protease DegS which is attached to the inner membrane and inactive under normal growth conditions. Protease activation is mediated by the C-terminal PDZ domain and can be induced by unfolded OMPs in vivo. Moreover, DegS has been implicated in the recognition of proteins marked by *ssra*-degradation signals [7]. However, activation can specifically be mimicked by the addition of 10 residue long synthetic peptides ending with C-terminal OMP sequences (OMP_{CT}) in vitro [7]. RseA destruction is continued by the integral membrane protease YaeL which is an orthologue of the mammalian Site-2 protease (S2P). This Zn-protease cleaves the substrate protein within or close to the plane of the membrane most probably at a cysteine residue [2,6]. The proteolytic two-step process finally leads to the release of the σ^E-factor which is otherwise tightly bound to the cytosolic domain of RseA. A release of σ^E results in increased transcriptional levels of genes under σ^E-control [8].

DegS is a 35 kDa protein with a N-terminal hydrophobic and membrane-bound α-helix followed by the protease and a single PDZ domain and the protein belongs to the HtrA (for high temperature requirement) class of proteins [9]. The structural architecture of these ATP-independent proteases are formed by the serine protease domain and a varying number of PDZ domains. Within this class of proteins DegS appears distinct as a membrane-bound enzyme of high substrate specificity. The only structures of homologous full-length proteins that have been determined so far are those of DegP from *E. coli* and HtrA2/Omi from mitochondria [10,11]. However, these proteins express a different mode of activation and function and their substrate specificity is diverse [12]. Notably, DegP can undergo a temperature-dependent switch between the chaperone and protease conformations but is structurally characterized only in its proteolytically inactive 'chaperone' conformation.

In our work we aimed to understand the structural basis underlying the activation of DegS by OMP_{CT} peptides

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generated from peptide libraries [5]. Using molecular replacement we determined the structure of the protein at 2.2 Å and we interpret data collected on a protein–peptide complex at 3.4 Å resolution.

2. Material and methods

2.1. Crystallization, data collection, phasing and refinement

Protein expression of DegS from *E. coli* missing the first 28 residues ($DegS_{\Delta N}$), purification and crystallisation was conducted as previously described [13]. Co-crystallization trials were performed with a peptide of DNRDGNVYYF sequence purchased from Thermo electron (Ulm, Germany).

Molecular replacement trials with a model generated using a superposition of trypsin-like protease domains (PDB entries: 1BE9, 1IHJ, 1PDR, 1QAV and 1UHP) were performed using the programs Phaser [14] and Molrep [15] and the unambiguity of the solutions was proven by radiation damage induced studies [16]. Two copies of the protease model were initially placed and refined with tight non-crystallographic restrains using Refmac [17]. Side chains were successively replaced by the DegS sequence to finally yield an electron density which made the missing PDZ domains visible. The PDZ domain of DegP was placed into the electron density and iterative cycles of model rebuilding with O and Refmac refinement at 2.2 Å led to an almost complete protein structure [17,18]. Solvent waters were added in F_{obs}–F_{calc} maps using the automated Arp Warp routine [19]. The final model comprises residues 37–354 according to the full-length protein sequence. Six residues (Tyr162, Leu218, Asn224, Asp225, Glu227 and His270) were modelled as alanine due to missing side chain densities and the geometry was finally checked with PROCHECK [20]. The solvent content V_M of the crystals was calculated as 55.2% [21]. The structural superposition of DegS with homologous proteins was performed using the program TOP (http://gamma.mbb.ki.se/~guoguang/top.html). All figures were prepared using the programs DINO [22] and Molscript [23].

Coordinates of the protein refined at 2.2 Å resolution have been deposited in the Protein Data Bank under the Accession No. 1TE0.

3. Results and discussion

3.1. Structure determination

The structure of the N-terminally truncated protein version of DegS from *E. coli* was solved by molecular replacement methods using coordinates of an homology model generated on the basis of trypsin fold conservation. The initial replacement trials returned only one monomer with significant high signals. The monomeric model consisting of 191 residues was refined at 2.2 Å and side chains were rebuilt according to the DegS sequence. However, at this stage we were not able to trace the missing parts of the protein. We therefore repeated our initial molecular replacement trials with the refined model and discovered a second protease monomer in the asymmetric unit. Both domains were further refined resulting in an electron density which allowed tracing of residues 37–354. Six undefined side chains of the otherwise well defined model were replaced by alanines and the structure has been refined to R/R_{free}-values of 24.7/29.5 and markedly high average B-factors of 55.4 with reasonable stereochemistry (see Table 1).

3.2. Architecture of the protease

Size exclusion chromatography indicates that both, full-length $DegS_{FL}$ and the truncated $DegS_{\Delta N}$ (abbreviated as DegS in the following), are trimeric and the membrane anchor (residues 1–28) appears dispensible for trimerization and function (data not shown). The state of oligomerization is different from the homologous DegP complex which is a hexameric soluble and ATP-independent chaperone [10]. We initially set out to structurally investigate $DegS_{FL}$ from *E. coli* including the N-terminal membrane helix. Unfortunately, the full-length protein gave only tiny crystals of 20–30 μm size which diffracted to not better than 6 Å using synchrotron radiation (data not shown). We therefore followed the analysis of an N-terminally truncated mutant protein.

Table 1
Data collection and refinement statistics

Data collection	
X-ray source/detector system	ID14-EH4/ADSC Quantum 4R
Wavelength (Å)	0.9393
Space group	I23
Cell constants	$a = 166.3$
Resolution range	30–2.2 (2.3–2.2)
No. of unique reflections	38296 (2399)
Redundancy	5.4 (4.9)
Completeness (%)	98.6 (90.9)
R_{merge}[a] (%)	0.048 (0.39)
$I/\sigma(I)$	15.8 (3.0)
Refinement	
Resolution range (Å)	30–2.2 (2.3–2.2)
No. of unique reflections	38296 (2399)
$R^{b}_{cryst}/R^{c}_{free}$ (%)	24.7/29.5 (25.6/33.6)
No. of protein atoms	4666
No. of waters	163
r.m.s.d. of bond length (Å^2)	0.025
r.m.s.d. of bond angles (°)	2.4
Average *B*-factor of protein atoms (Å^2)	55.4
Ramachandran plot statistics, residues	
In most favoured region	81.9%
In add. allowed regions	13.2%
In gen. allowed regions	1.9%
In disallowed regions	3%

Values in parentheses refer to the highest resolution shell.
[a] $R_{merge} = \sum_{\text{unique reflections}}(\sum_{i=1}^{N}|I_i - \bar{I}|)/\sum_{\text{unique reflections}}(\sum_{i=1}^{N} I_i)$, where N represents the number of equivalent reflections and I the measured intensity.
[b] $R_{cryst} = \sum|F_{obs} - F_{calc}|/\sum|F_{obs}|$.
[c] R_{free} was calculated using 5% randomly selected reflections.

The overall structure of monomeric DegS is shown in Fig. 1(a). The protein consists of two globular structural domains: a serine protease (residues 37–251) and a PDZ domain (residues 252–354). The structure of DegS comprises 11 α-helices and 18 β-strands forming a predominantly well-defined structure expressing a mixed α/β-fold. Residues of the catalytic triade are buried in the cleft of two β-barrels formed by β1–β6 and β7–β12 and reside approx. 30 Å above the inner membrane border (see Fig. 1(a)). The protease domain adopts a fold similar to members of the chymotrypsin family with a 1.6 Å r.m.s. deviation to the closest neighboring model 1AGJ for 124 aligned Cα atoms. According to homology DegS may be further classified into the class of Htra proteins comprised by members with the general architecture of a trypsin-like protease fused to the PDZ sensor or substrate binding domain [9]. DegP the only structure of a bacterial Htra full-length homolog displays a slightly higher structural conservation to DegS than HtrA2/Omi the mammalian homolog from mitochondria (HtrA2 in the following), the second full-length structure available (see Figs. 3(d) and (e)). These structural similarities are expressed in the r.m.s. values of 1.15 (for 151 residues) and 1.18 (for 158 residues) for the protease and 1.47

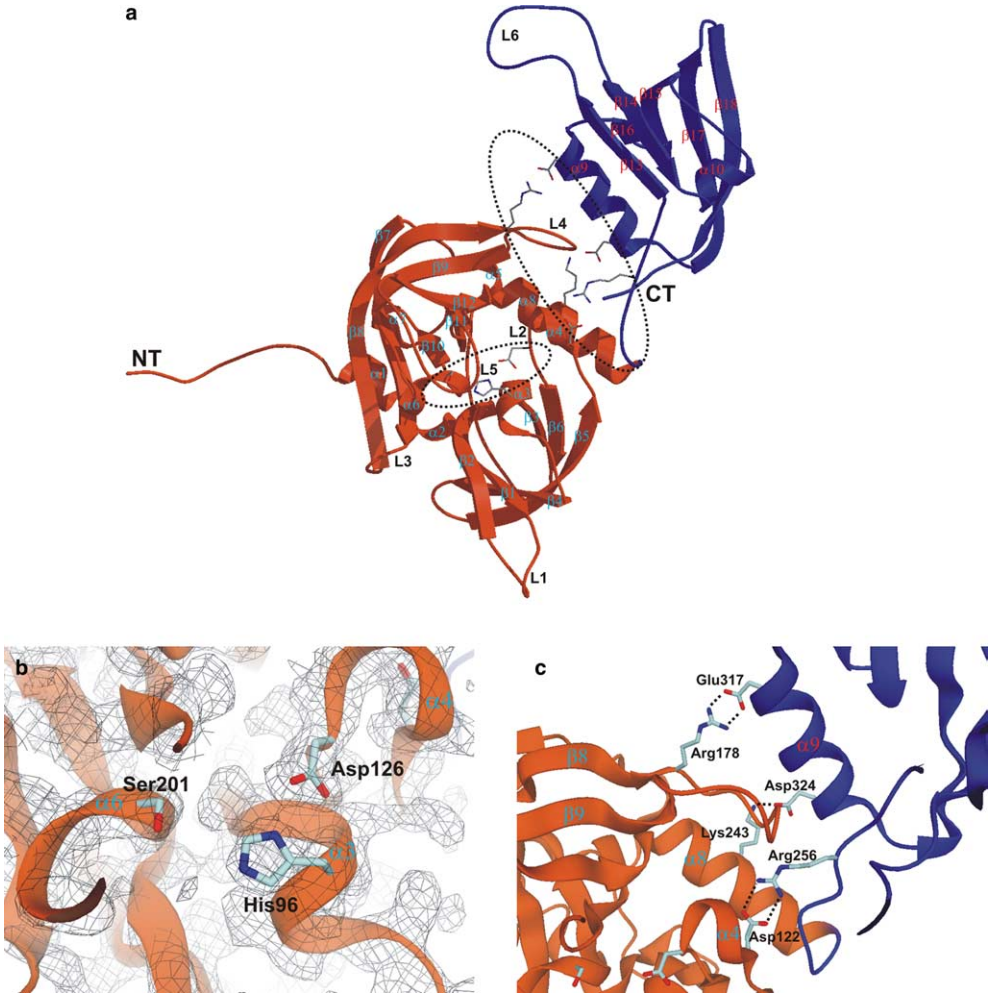

Fig. 1. Crystal structure of the substrate sensor DegS from *E. coli*. (a) Schematic representation of DegS structure showing the protease domain in brown and the PDZ domain in blue. N- and C-termini (NT, CT), the β-strands (β1–β18), α-helices (α1–α9) and loop structures (L1–L6) important for the function are indicated. Residues of the catalytic triade and the domain interface are highlighted and marked by dotted circles. (b) Close-up of the $2|F_{obs} - F_{calc}|$ electron density map calculated around the protease active center and contoured at 1.2σ. The residues forming the catalytic triade (His96, Asp126 and Ser201) are marked. (c) A close-up view of the intramolecular hydrophilic contacts between protease (brown) and PDZ (blue) domain. Important residues of the interface are marked with numbers according to the DegS sequence.

(for 72 residues) and 1.3 (for 68 residues) for the PDZ domain, respectively (see Figs. 3(a) and (b)).

There are structural features in the protease domain which differ significantly between DegS and its structural homologs. The L1 loop, i.e., of DegS connecting two anti-parallel β-strands β1 and β2 resides on the opposite side to the N-terminus of the protein trimer (Figs. 1(a) and 3(a)). This loop of 10 residues with high flexibility (B-factors > 80) is identical in length to the trimeric HtrA2 but contains an insertion element of 40 residues in DegP. This feature in DegP is the underlying structural basis for hexamerization and formation of a hydrophobic chamber for protein folding and rescue of misfolded proteins (see sequence alignment in Fig. 2) [10].

The active site triade of DegS formed by residues His96, Asp126 and Ser201 shows residues well defined in the electron density (see Fig. 1(b)). A superposition of the homologous residues from DegP, HtrA2 (for DegP and HtrA2 proteolytically inactive SA mutants were used for crystallisation) and trypsin showed a good match in the relative position of only two residues, the aspartate and histidine (see Fig. 3(c)). Surprisingly the superposition of the active site serines expresses a remarkable deviation from the ideal position defined by the conserved trypsin fold. Such a deviation from the ideal may critically influence the activity of DegS and DegP via formation of the oxyanion hole [10]. These significant differences in active site residues are also evident from the superposition of secondary structure elements (see Figs. 2 and 3(a)). Whereas loop L2 and α3 are well aligned for His96 and Asp126 they deviate obviously in helix α6 of DegS which holds the serine position, resulting in a deep embedding into the active site of the DegS structure (see Figs. 3(a) and (c)).

In DegP a loop triade LA*-L1 and L2 blocks the accession to the catalytic site with LA* derived from a second monomer. Although the active site of DegS is occupied by only two of these loops, L3 (L1 in DegP) and L5 (L2 in DegP), the accessibility from the L5 side is even more impeded by the insertion of three additional residues into L5 (Glu227, Thr228, Pro229, see Figs. 2 and 3(a)). Interestingly, the active site of HtrA2 is also shielded by extended loop structures and the PDZ domain in particular but the L5

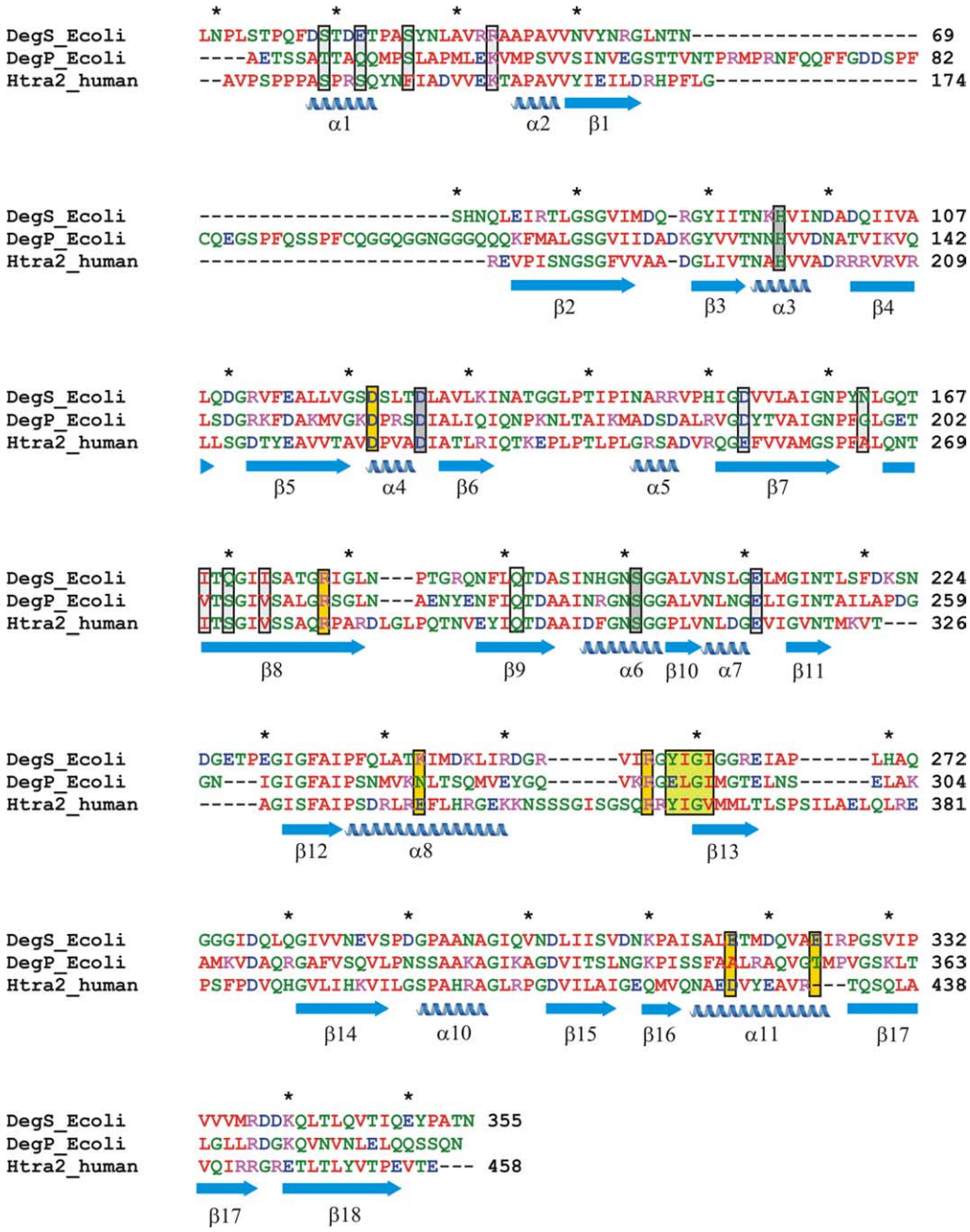

Fig. 2. Sequence alignment of DegS and the structural homologs DegP and Htra2/Omi. Secondary structure elements are indicated below the alignment. The catalytic triade residues are boxed and marked in dark grey, the OMP_{CT} peptide binding site (residues 258–261) is marked in green, the residues of the intradomain interface are marked in yellow. Residues boxed and not colour shaded contribute to interdomain interactions.

homolog loop i.e., is truncated (see Figs. 3(a) and (e)) [11]. Nevertheless, in contrast to DegS the mitochondrial protein exhibits an increased proteolytic activity after removal of the PDZ domain whereas activity of DegS and DegP after PDZ deletion is abolished [5,12]. Given these obervations its tempting to speculate that a combination of the spacial deviation of a single residue at the serine position from an ideal 'trypsin-like' location and the particular loop structure characteristics surrounding the active center may keep DegS (and also DegP in the 'chaperone' form) in the inactive state described.

3.3. *Architecture of the PDZ domain and the intradomain interface*

In an overlay of the three PDZ domains from DegS, DegP and HtrA2 shown in Fig. 3(b) the most significant changes occur within loop L6 that connects the antiparallel β13 and β14 strands. This loop with high residual B-factors (B-factors > 80) may – in this extended conformation – weakly interact with the adjacent protease domain of the neighboring molecule (closest distance 4.1 Å). While the overall fold of the PDZ domain is highly conserved, the proteins differ substantially in the relative orientation of the intradomain arrange-

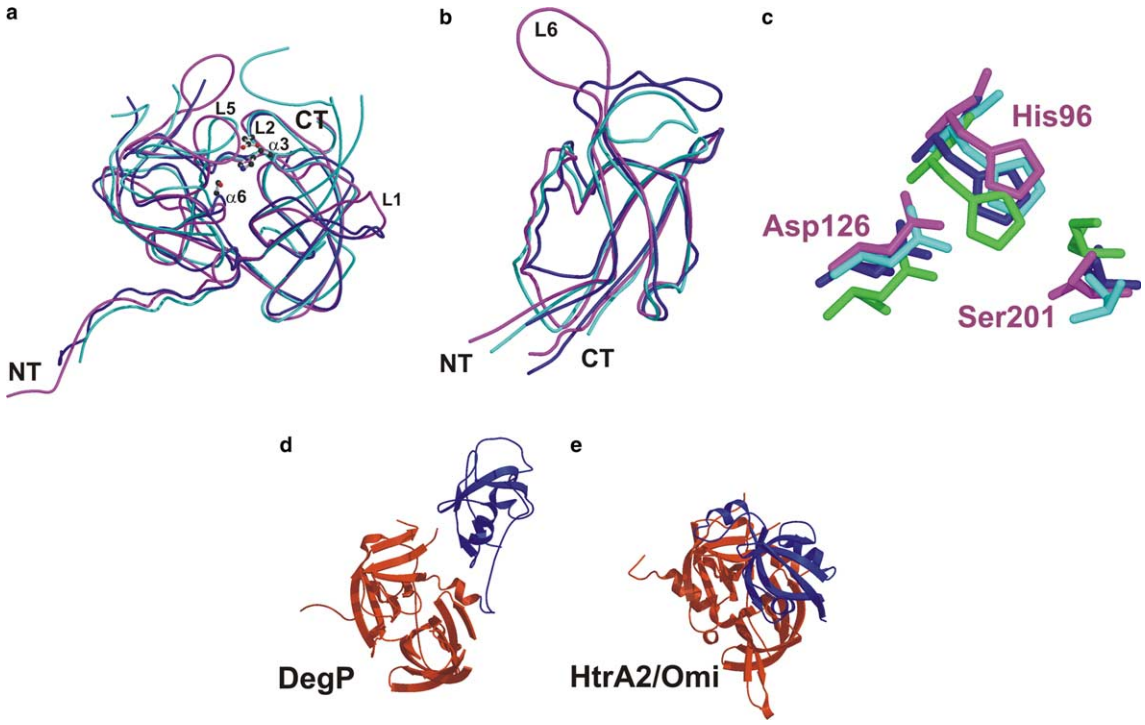

Fig. 3. Structural comparison of Htra proteins. (a) Structural alignment of the protease backbone atoms. N- and C-termini (NT, CT), loop5 (L5) and resdiues of the catalytic triade are assigned in ball and stick for clarity. The following colour code was used: DegS is magenta, DegP is cyan and HtrA2/Omi is coloured in blue. (b) Structural alignment of the PDZ domain with the same colour code used in (a). Selected secondary structure elements are marked. (c) Overlay of the active site residues from DegS, DegP, HtrA2/Omi and trypsin (in green). Residue numbers are assigned with respect to the DegS sequence. (d) Crystal structure of DegP with the protease domain superimposed onto DegS and the domain colour code according to the DegS structure in Fig. 1(a). (e) Crystal structure of HtrA2/Omi with the protease domain structurally aligned to DegS and the same colour code as for figure (d).

ment. The ultimate α8 helix of the protease and β13 of the PDZ domain are covalently connected by a 9-residue long linker (residues 251–259) which is stabilized by a salt bridge between Asp122 and Arg256 at the boundary of the domain interface (see Fig. 1(c)). This particular linker region is further extended in HtrA2 by six additional residues but flexible and thereby not visible in the crystal struture (see alignment Fig. 2). In DegS the intradomain boundaries are more tightly connected and locked to each other by the formation of two additional salt bridges located between residues Lys243 and Asp324 and Arg178 and Glu317. The three ion pairs are conserved only for residues Asp122 and Arg256 (see Fig. 2) in DegP and HtrA2. Moreover, in DegQ the second member of a membrane-bound Htra-protein protease in the bacterial periplasm homologous residues are also missing implying another mode of activation.

The size of the interfacial area is comparable to many protein–protein interactions of moderate affinity with 1450 $Å^2$ of solvent accessible area buried by the formation of the interface. Interestingly, the same orientation of the PDZ relative to the protease domain was independently observed in crystals with different packing constraints (space group P23, data not shown) and corroborates this assembly to be conserved. Average B-factors of 66 calculated for the PDZ domain are only slightly higher than those of the protease (50) indicating a stable arrangement between both domains.

In DegS the PDZ domain is kept in an orientation that does not restrict access to the catalytic site from the top of the trimeric protein as observed for HtrA2 and DegP [10,11]. However in the oligomeric structure loop L6 points to the neighbouring protease domain of the adjacent monomer thereby blocking direct lateral access of a putative substrate protein such as RseA towards the active center (see Fig. 5(b)). In the DegP crystal structure of the 'chaperone form' the corresponding PDZ1 domains of two symmetry related monomers occur in differing 'open' and 'closed' orientations (see Fig. 3(d) for one of these two conformations). In the latter access towards the active center is prevented. Their distinct mobility with unusually high B-factors (>150) indicates a proposed mechanistically important flexibility which may – in this case – allow both, substrate binding at the outer surface of the protein cavity and the formation of a closed and hydrophobic chamber for substrate processing. It remains speculative whether the two conformations of the protease reflect physiological conformations since the structure was determined in a substrate-free form and the conformational shifts may have resulted from crystal packing constraints [10]. In HtrA2 the corresponding interactions are primarily formed by hydrophobic stretches on both, the protease and the PDZ surface. By these interactions the PDZ domain completely blocks access to the active center of the protease through van der Waals contacts (see Fig. 3(e)). Strikingly most of these residues are conserved on the protease surface of both bacterial homologs but the counterparts are absent on the PDZ domain (Fig. 2).

3.4. The trimeric interface

Homotrimerization of $DegS_{FL}$ is exclusively mediated by the protease domain where two independent stretches of residues account for the overall stabilization. The first interface between the N-terminal part of one molecule is formed by an elongated tail whose conformation is stabilized by interactions to the adjacent monomer via H-bonds between Ser39 and Glu211, Ser46 and Asp153 and a salt bridge between Glu42 and Arg53 (see Fig. 4(a)). Only Glu211 and its counter residue Ser46 is conserved among DegP (here replaced by threonine) and HtrA2 whereby a remarkable general contribution of the N-terminus to the overall stability of the trimeric complex appears questionable. However the importance of N-terminal residues for trimerization and function in HtrA2 was emphasized earlier by the observation that truncated mutants of HtrA2 (N-terminal Δ16 mutant) loose the ability of trimer formation and activity [11] although the interactions in HtrA2 differ from DegS as aromatic side chains are exclusively involved.

A second interface was discovered by the analysis of conserved residues mapped on the monomer surface according to sequence homology based on sequences of DegS, DegP and HtrA2 (see Figs. 2 and 4(b)). Surprisingly only two of the residues detected by sequence homology and displayed on the surface, Ile172 and Gln191, are within a distance of reasonable attracting interactions and contribute to additional contacts most of which are of van der Waals nature. Residues of this patch which exclusively map to the second β-barrel of the protease domain (β7–β12) are predominantly comprised by hydrophobic and small amino acids which may contribute to the maintenance of the protein backbone and thereby preserving important folding restraints.

A putative model of a full-length DegS homotrimer shown in Figs. 5(a) and (b) was generated based on the fusion of a typical membrane helix to the N-terminus of the crystals structure. Although the first nine residues connecting the anchoring helix with the periplasmic domain are missing in this model the arrangement of three membrane-embedded helices with helix–helix distances of approx. 50 Å suggests a mechanically stable insertion into the inner membrane and high stability towards the lateral membrane pressure (see Fig. 5(a)).

3.5. Putative mode of activation – mechanistical considerations

The DegS protease initiates a proteolytic cascade which requires the activation by the C-termini of unfolded OMP proteins in vivo or consensus peptides representing these C-termini sequences in vitro [5]. We therefore set out to co-crystallize the protein in the presence of a OMP_{CT} consensus peptide in order to gain insight into the specific mode of protease activation. Co-crystals were yielded under the almost identical conditions with preserved cell constants but never diffracted to better than 3.4 Å resolution and did not allow us to trace structural changes unambiguously that may have occurred upon binding. Nevertheless, rigid body refinement calculations with protease and PDZ as independent domains demonstrated the relative orientation and connection being preserved as in the peptide-free protein. Moreover, experiments of limited proteolysis were carried out using mixtures of DegS and either subtilisin or proteinase K at different relative concentrations, both in the presence (0.3 mM peptide) and absence of the activating peptide. Careful analysis of degradation products yielded by these proteases using SDS-gels did not reveal any difference visible in the proteolytic finger print of the complexed and uncomplexed form. An indication of this arrangement to be rigid is therefore underlined by the the fact that peptide binding did at least not remarkably influence the surface accessibility of the protein complex towards the proteases tested (data not shown). One can therefore tentatively conclude that binding of the peptide does not induce a remarkable change in the intradomain interface. It is unlikely that the peptide foiled to bind during co-crystallisation under conditions which were not dissimilar to those used in [5] for peptide binding studies.

Negligible changes within the protease–PDZ interface are somewhat contradictary to a mechanism proposed by Walsh et al. [5] based on biochemical and NMR studies of the isolated PDZ domain performed in the absence and presence of the activating peptide. In this paper the authors addressed the variances mainly based on HSQC assignments of the PDZ domain only and assign these changes to significant conversions within the domain interface. Under the experimental conditions chosen by the authors the influence of peptide binding on the neighboring protease domain was neglected [5].

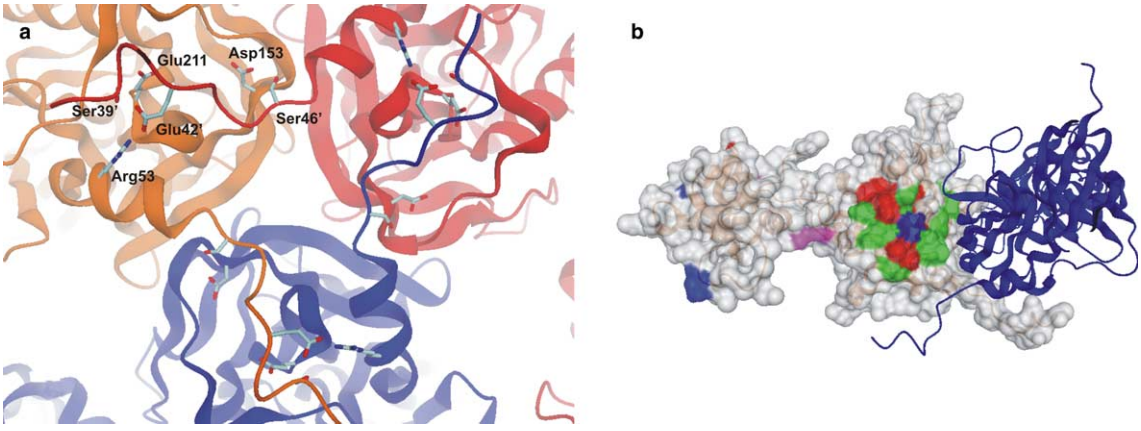

Fig. 4. Conserved residues involved in trimerization. (a) Close-up of the trimerization zone in DegS. The important residues of the interdomain interface between two adjacent monomers are marked with numbers. (b) Surface representation of monomeric DegS with a ribbon model of the adjacent monomer in blue displayed at the monomer–monomer protein interface. Conserved residues are coloured according to the alignment of Fig. 2: hydrophobic residues are red, acidic residues are blue, basic residues are magenta and amphipatic residues are green.

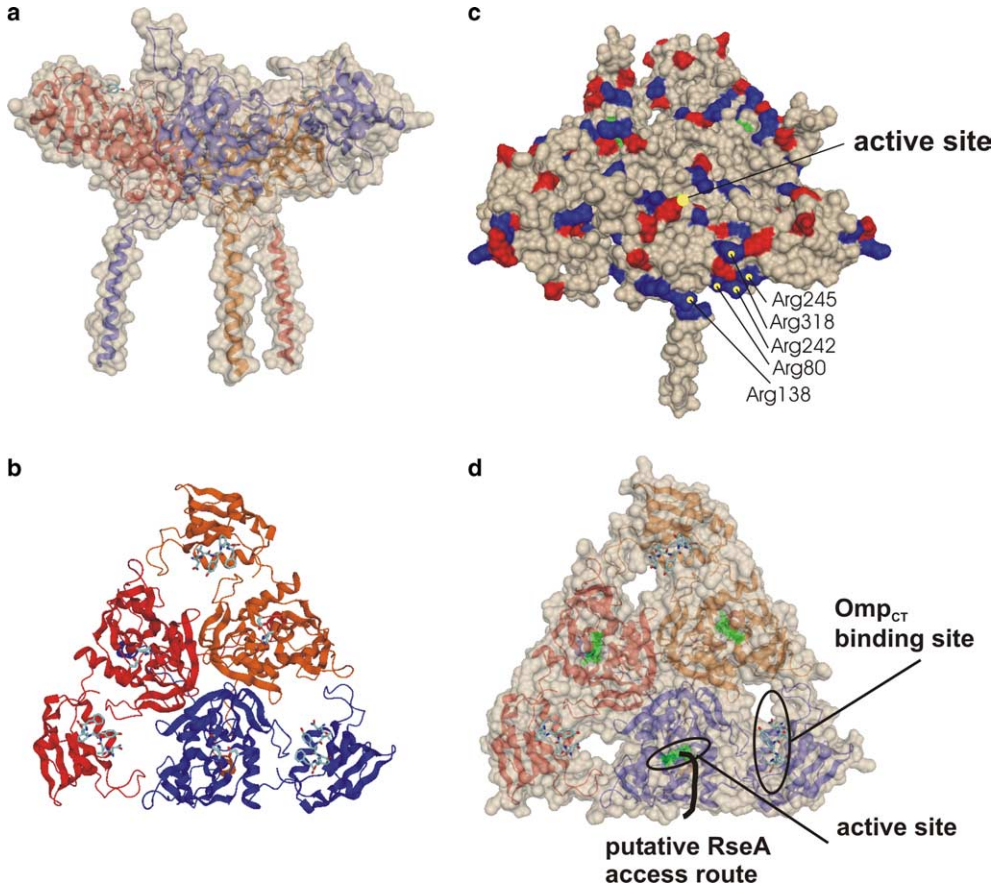

Fig. 5. Model of activation. (a) Side view of a surface calculated of a DegS model including the membrane helices. The three monomers were colour coded in brown, red and blue. The model was constructed on the basis of the DegS structure and combined with helix E of the membrane protein halorhodopsin (PDB-entry: 1E54). (b) Trimeric ribbon model of DegS as topview. The catalytic triade and peptides representing the last five residues of the high-affinity peptides published in [5] are modelled to the substrate binding groove of the PDZ domain. (c) Surface representation of trimeric DegS including the membrane-helices. The acidic residues on the surface are marked in red whereas basic residues are marked in blue. The arginine ladder consisting of five arginines and located on the outermost surface is marked with the adjacent residue numbers. (d) Putative mode of activation. The important structural features of DegS are marked. The Omp_{CT} peptide binding site is located in the vicinity of the intradomain interface. Peptide binding can induce a structural rearrangement within the protease domain leading to stuctural changes and activation of the catalytic triade. A putative access route of the RseA substrate protein towards the active center is marked in black.

Assuming a fixed orientation of the PDZ domain under activated conditions our observations may shed light on physiological observations of bacterial strains carrying the *degSΔPDZ* protein in an unaltered genetic background. Under these conditions a putative protection of the active site by PDZ is omitted and an activated situation would be mimicked. PDZ-truncated protein mutants of DegS however do not exhibit a significantly increased basal σ^E activity even under conditions where the protein is overexpressed. Moreover increased expression of cytochrome fusions carrying the last 50 OmpC residues and ending with different terminal sequences failed to induce σ^E induction in *ΔdegS* strains expressing plasmid-borne *degSΔPDZ* [5]. To conclude: the presence of the PDZ domain in a locked conformation in DegS appears critically for the proper enzymatic function. Noteably, similar experiments were also performed for *HtrA2ΔPDZ* but led in this case to an uncontrolled degradation of substrate proteins [11].

An analysis of the charge distribution on the DegS surface reveals a cluster of five arginine charges (Arg88, Arg146, Arg250, Arg253 and Arg326) on the interfacial surface between protease and PDZ domain. The arginine residues form a line of positive charges originating at the membrane surface next to the putative location of the intergral membrane helix of the adjacent protein monomer and ending at the upper plateau of the trimeric protease (see Fig. 5(c)). The charges almost specifically follow the closest access route towards the catalytic center that a membrane-bound substrate protein can take to become degraded. RseA contains a significant number of negatively charged residues, specifically glutamates and stretches of glutamines close to the section facing the transmembrane part [5]. These residues may interact with charges exposed on the DegS surface. Moreover, it has been reported that the kinetics of RseA destruction by DegS are critically dependent on the orientation and access of the substrate protein towards the protease which occurs much faster if both proteins are oriented in the same direction [5]. Its therefore tempting to speculate that the initial interactions between substrate and protease may be specifically formed by both, unspecific hydrophobic interactions within the membrane plane and specific hydrophilic interactions through salt bridges and hydrogen bonds on the outermost protein surface.

What is the mode of activation by OMP_{CT} peptides in DegS? The structural characteristics of the catalytic triade and the surrounding environment together and the observation of a locked intradomain arrangement imply the following model: activation of the protease can be accomplished by a physical interaction of C-terminal OMP protein sequences with the PDZ-domain. Additional binding interactions are simultaneously formed with surface exposed loop stuctures of the protease domain which may be located close to the peptide binding groove and can then induce a conformational shift in both, the catalytic serine residue and in the adjacent loops, probably leading to loop rearrangements. Upon activation access of the substrate protein RseA is enabled. A possible direct influence of RseA on the activation process has not yet been studied and it remains speculative whether binding of the RseA substrate to DegS accounts for additional activating effects. Such a substrate-based mechanism of activation was already proposed for the temperature-induced proteolytic activation of DegP. In this scenario the PDZ domain carries out a function as to a mechanically linked adapter protein for substrate recognition and inhibition or activation of the proteolytic domain [10].

During revision of the present manuscript a broadly similar structure of DegS was published by others [24].

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