

Structure of the month

PNAS 2012 108(26):10490-95.

The *Legionella* HtrA homologue DegQ is a self-compartmentizing protease that forms large 12-meric assemblies

Robert Wrase^a, Hannah Scott^a, Rolf Hilgenfeld^{a,b,c,1} and Guido Hansen^a

^a *Institute of Biochemistry, Center for Structural and Cell Biology in Medicine, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany*

^b *Laboratory for Structural Biology of Infection and Inflammation, c/o DESY, Building 22a, Notkestr. 85, 22603 Hamburg, Germany*

^c *Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Rd., Shanghai 201203, China*

¹ Corresponding author: Rolf Hilgenfeld, Institute of Biochemistry, Center for Structural and Cell Biology in Medicine, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany

E-mail: hilgenfeld@biochem.uni-luebeck.de

Phone: (+49)451-500-4060, Fax: (+49)451-500-4068

Abstract

Proteases of the HtrA family are key factors dealing with folding stress in the periplasmic compartment of prokaryotes. In *Escherichia coli*, the well-characterized HtrA-family members DegS and DegP counteract the accumulation of unfolded outer-membrane proteins (OMPs) under stress conditions. Whereas DegS serves as a folding-stress sensor, DegP is a chaperone-protease facilitating refolding or degradation of defective OMPs. Here we report the 2.15-Å resolution crystal structure of the second major chaperone-protease of the periplasm, DegQ from *Legionella fallonii*. DegQ

assembles into large, cage-like 12-mers which form independently of unfolded substrate proteins. We provide evidence that 12-mer formation is essential for the degradation of substrate proteins but not for the chaperone activity of DegQ. In the current model for the regulation of periplasmatic chaperone-proteases, 6-meric assemblies represent important protease-resting states. However, DegQ is unable to form such 6-mers suggesting divergent regulatory mechanisms for DegQ and DegP. To understand how the protease activity of DegQ is controlled, we probed its functional properties employing designed protein variants. Combining crystallographic, biochemical and mutagenic data, we present a mechanistic model that suggests how protease activity of DegQ 12-mers is intrinsically regulated and how deleterious proteolysis by free DegQ 3-mers is prevented. Our study sheds light on a previously uncharacterized component of the prokaryotic stress-response system with implications for other members of the HtrA family.

Table S3: Crystallographic data

	DegQ	DegP ¹	DegP ² -DPS2	DegP ³ -D1
EMBL ID	Q9U2	Q9U3	Q9U4	Q9U5
Open groups	89 (1.04)	85 (1.04)	80 (1.04)	80 (1.04)
Clash with N, O, C (Å)	136.76, 136.76, 137.08	139.63, 139.63, 138.96	143.89, 143.89, 143.89	137.11, 137.11, 138.96
Resolution (Å) ^a	39.86, 2.17 (2.17), 2.17	39.86, 2.16 (2.17), 2.16	39.86, 2.16 (2.17), 2.16	40.86, 2.06 (2.07), 2.06
Subunit content (Deg)	62	61	61	60
Molecular mass	0	0	1	0
R _{int} (%)	0.10	0.10	0.10	0.10
R _{free} (%) ^b	47.0 (4.6)	57.0 (5.7)	57.0 (5.7)	4.0 (0.7)
Mean I/σ(I)	10.1 (2.0)	8.4 (2.0)	8.3 (2.0)	10.0 (2.0)
Cross-correlation (%) ^c	95.1 (0.7)	90.7 (0.6)	90.8 (0.6)	90.0 (0.6)
Multiplicity ^d	2.0 (2.0)	2.0 (2.0)	2.0 (2.0)	2.0 (2.0)
Unique reflections ^e	117,877 (1,046)	41,881 (3,675)	36,910 (3,261)	89,643 (1,966)
Average resolution (Å)	6.34	6.08	6.16	5.63
R _{int} (I/σ(I)) ^f	11.36 (2.06)	12.46 (2.05)	12.19 (2.05)	10.11 (2.04)
Average B-factor (Å ²)	62.7	72.7	72.7	56.2
Non-hydrogen residues ^g	37,076, 2.17% (2.16)	33,685, 2.0% (2.0)	30,474, 2.0% (2.0)	37,085, 1.97% (1.97)

^aResolution in parentheses refers to the highest resolution shell.

^b $R_{free} = \frac{\sum |F_o - \langle F_o \rangle|}{\sum |F_o|}$ - R_{free} is the R-value for 5% of the reflections excluded from refinement.

^cperfectly agrees, different regions, Additional regions

Table 1.

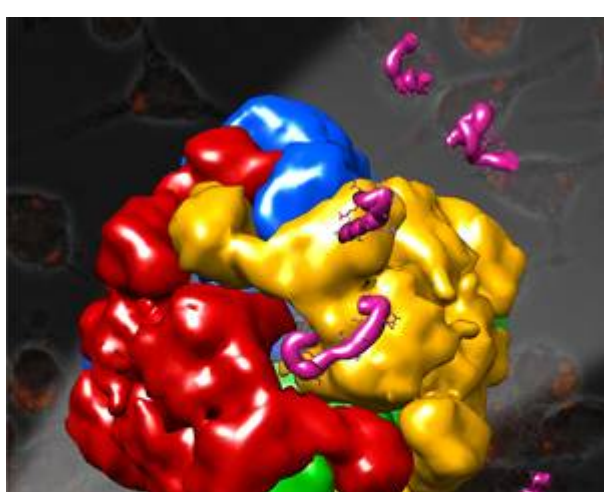




Figure 1.

DegQ from Legionella fallonii is a chaperone-protease responsible for protein quality control in the bacterial periplasm. A DegQ 12-mer composed of 4 trimers (red, blue, yellow, green) is shown in surface representation. To visualize the proteolytic activity of the 12-mer, fragments of a misfolded substrate molecule (purple) undergoing degradation have been manually added. Macrophages infected with Legionella bacteria are shown in the background.

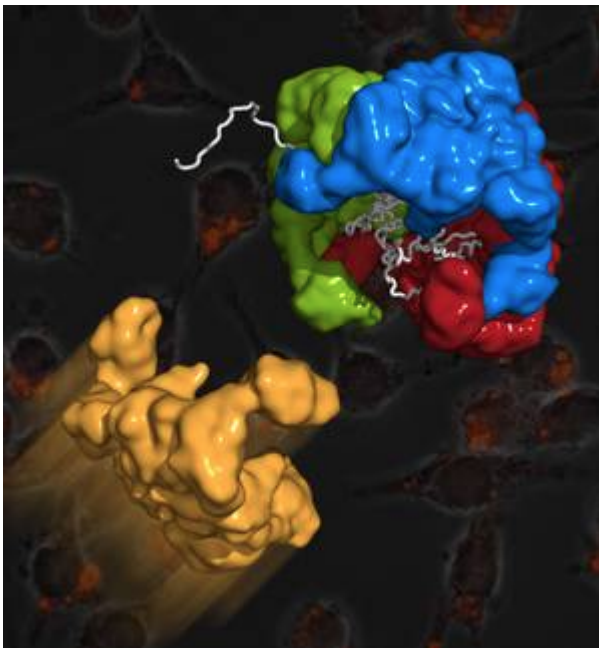


Figure 2.

Model of a DegQ molecule in the process of encapsulating a substrate molecule. DegQ trimers (red, blue, yellow, green) are shown in surface representation and a substrate molecule as ribbons (white).

Last update: 15.01.2013, responsible: Uwe Müller, stellvertretend: Manfred Weiss