Whopping Cough : Role of DegP Chaperone on the secretion of the virulence factor FHA via TPS pathway

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TPS PATH – ANR MIME 2007

Structure-based functional studies of the TPS pathway in Gram-negative bacteria : deciphering molecular aspects of protein transport in the TpsB/Omp85 superfamily.

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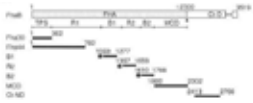
Objectives

Whopping cough is a bacterial respiratory infection caused by *Bordetella pertussis*, a gram negative bacillus. Despite the use of whole cell pertussis vaccine, the occurrence of whopping cough increases progressively *de novo*. Worldwide, as many as 45 million newly infected people/year can be estimated and 300.000 death/year are reported. The secretion of virulence proteins is an important feature of bacterial pathogenesis. The Two-Partner Secretion (TPS) systems have been identified in a number of important pathogens, including *Bordetella pertussis*, *Haemophilus influenzae*, *Neisseria meningitidis* etc. TPS pathway is devoted to the secretion of large proteins serving mostly as virulence factors and collectively called « TpsA » proteins, by their specific « TpsB » transporters across the outer membrane. In our project, we propose to study the molecular mechanisms of protein transport in the FHA-FhaC system in *B. pertussis* as model for the TPS pathway in bacteria. In the work presented here, we have focused on the periplasmic step of FHA secretion. The filamentous haemagglutinin (FHA) is a major adhesin of *B. pertussis*. As a prototypic TpsA protein, the 230kDa FHA forms a long β-Helix and carries a conserved N-ter « TPS » domain that mediates specific interactions with its transporter FhaC. According to our experimental data, FHA doesn't fold in the periplasm and FhaC recognizes a non native conformation of the TPS domain. FHA thus may need to be protected from aggregation, misfolding and proteolytic degradation raising the issue of putative periplasmic chaperones in the secretion pathway. Two were studied : Par27 and DegP.

Results

1- FHA structure & constructs



A number of FHA fragments were produced. Among them, Fha30 and Fha44 are competent for secretion.

2- Periplasmic chaperones studied

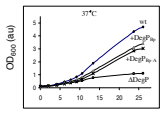
A- Par 27 (peptidyl prolyl isomerase) is not essential for FHA secretion

Par27 interacts with denatured Fha30 *in vitro*, prevents its aggregation and partially protects it against proteolytic degradation *in vitro*.
But : *B. pertussis* strain Δ Par27 grows slightly slower than wt but is not defective for FHA secretion. (Hodak *et al*, JMB 2007)

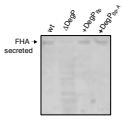
B- DegP

DegP - first identified in *E.coli* - is a periplasmic protein that belongs to the HtrA (HighTemperature requirement) family.
HtrA members have common features : a N-ter serine protease domain and C-ter PDZ domain(s). DegP exhibits both chaperone and protease activity. It's a quality control protein in the periplasm.

$DegP_{Bp}$ contains one protease and two PDZ domains.



Inactivation of DeP_{Bp} markedly slows down bacterial growth and affects both FHA secretion and membrane integrity (leakage of cytoplasmic and periplasmic proteins).

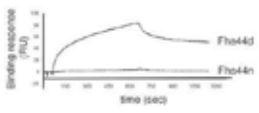


$DegP_{Bp}$ facilitates FHA secretion

3-$DegP_{Bp-A}$/FHA interactions *in vitro*

DegP from *B. pertussis* was purified in recombinant form from *E. coli*. A protease-inactive form was also produced ($DegP_{Bp-A}$)

SPR:



$DegP_{Bp-A}$ interacts only with unfolded forms of FHA

Affinity of $DegP_{Bp-A}$ for FHA domains:

	Fhe30	Fha44	B1	R2	B2	MCD	Ct ND
K_d (μM)	0.14±0.07	0.53±0.11	0.23±0.03	0.36±0.08	ND	ND	0.89±0.19

$DegP_{Bp-A}$ recognizes domains with β-Helical structure

4-$DegP_{Bp}$ activities

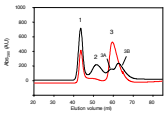
DegP's chaperone activity was tested by determining its ability to prevent aggregation of a denatured substrate following dilution of the denaturant. DegP's protease activity was tested by incubating DegP with native or denatured proteins. Degradation was followed on SDS-PAGE gels after silver staining.

FHA is a substrate for $DegP_{Bp}$ chaperone and protease activities *in vitro*

Baud *et al*, 2009. Mol. Microbiol. 74(2) : 315-29
Clantin *et al*, 2010. J.Struct. Biol. 169(3) : 253-65

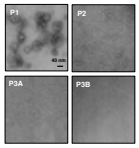
5- Characterization of the oligomeric forms of $DegP_{Bp}$

SEC coupled to MALLS for mass determination.



(i) $DegP_{Bp}$ (red line) consists in two oligomeric forms : P1 >1MDa and P3 (trimer).
$DegP_{Bp-A}$ (black line) consists in four oligomeric forms :P1 >1MDa, P2 (12-mer), P3A (hexamer) & P3B (trimer).
The same forms were found in *B. pertussis*.
(ii) Several *E. coli* envelope proteins (OmpF, OmpA…) co-purified in low amount with $DegP_{Bp-A}$ 12-mer and with the large DegP forms.

Electronic microscopy



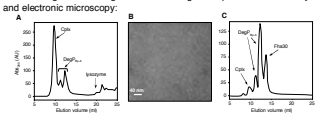
(i) $DegP_{BP-A}$ forms dodecamers. As shown for *E. coli* DegP, these presumably represent substrate sequestering cages (Krojer *et al.*, 2008; Jiang *et al.*, 2008).
(ii) The >1 MDa complexes are large vesicular structures suggesting association of $DegP_{Bp}$ with lipids. TLC and mass spectrometry identified PE and PG but no LPS in these complexes, indicating that they represent cytoplasmic membrane-associated $DegP_{Bp}$. Extraction methods showed that $DegP_{Bp}$ is a peripheral membrane protein.

6- Activities of the various $DegP_{Bp}$ forms

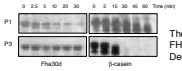
A- By SPR, the 12-meric and membrane-associated forms displayed higher affinities for Fha44 than the 3-mers. In contrast, the trimers had higher affinities for globular, model substrates (such as lysozyme).

Interactions are strongly dependent on the substrate.

B- Rearrangement of DegP trimers into larger complexes monitored by SEC and electronic microscopy:



C- Protease activity:



The trimers degraded β-casein but not FHA. In contrast membrane associated $DegP_{Bp}$ degraded both types of substrates.

Unlike globular protein substrates (i.e lysozyme) of DegP, the repetitive FHA protein does not efficiently trigger the rearrangement of soluble $DegP_{Bp}$ trimers to the high-protease-activity 12-meric form.

D- Chaperone activity:
The trimers prevented lysozyme aggregation. Membrane bound DegP, but not the trimers, associated with Fha44 causing a significant increase in complex size.

E- *In vitro* reconstitution of lipid-associated DegP:
In vitro, soluble $DegP_{Bp}$ trimers have affinity for lipids, in contrast to 12-mer. The complex formed has an EC elution profil similar to that of P1 and the same chaperone and protease activities.

Association with lipids modifies the properties of $DegP_{Bp-A}$ trimer.

Baud *et al*, 2011. Mol. Microbiol. In Press

Conclusion

In *Bordetella pertussis*, $DegP_{Bp}$ facilitates the secretion of FHA, a long β-helical adhesin that passes through the periplasm in an extended conformation. In this work, we shown that $DegP_{Bp}$ exists as soluble trimers and a membrane-associated form and we characterized these various oligomeric forms. Different substrates interact differently with the distinct forms of $DegP_{Bp}$, and membrane-associated $DegP_{Bp}$ has high affinity for non-native FHA. Unlike more globular substrates, FHA does not efficiently mediate the rearrangement of trimers into proteolytically active, short-lived dodecamers. In contrast to these dodecamers, membrane-associated $DegP_{Bp}$ is not committed to substrate degradation, although it is proteolytically competent. In *B. pertussis,* membrane-associated $DegP_{Bp}$ thus represents a specific functional form serving as a holding chaperone for client proteins including FHA. If FHA secretion is impaired, membrane-associated $DegP_{Bp}$ participates in its degradation. Such a dual-purpose form of DegP is appropriate to handle substrates unsuitable to be sequestered in cages or non-folded secretory proteins that must not be degraded.

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