(1) Submission ID#1527283

Convenient purification of recombinant type IV pilins using engineered signal peptidase I cleavage

---

Author(s)

Justin Applegate, n/a

PhD Candidate

University of Washington - Department of Biochemistry

Background

The type IV pilus (tfp) is an essential virulence factor of pathogenic Neisseria. Tfp are polymeric fibers used by Neisseria sp. That mediate adhesion to the host, bacterial movement via twitching motility, and uptake of extracellular DNA for natural transformation. The majority of the fiber is made of the major pilin protein, PilE. PilE is an integral transmembrane protein with a N-terminal α-helix incorporated into the inner membrane and a C-terminal globular domain located on the periplasmic side. When formed into the pilus fiber, the α-helix makes up the core of the fiber, while the globular domain is exposed on the fiber's surface. There are several proteins which share the structural motif of the major pilin, but are incorporated into the fiber in much lower amounts. The five minor pilins – PilH, PilI, PilJ, PilK, and PilL – are essential for efficient pilus extension and are believed to form a tip located cap that primes pilus biogenesis.

Aim/Methods

Minor pilins are integral membrane proteins bound to the inner membrane of gram-negative bacteria, and typically have one or two disulfide bonds. Purifying them can be technically challenging. Signal peptidase I (SPI) recognizes and cleaves periplasmic proteins containing an "Ala-X-Ala" amino acid motif. Plasmids were constructed which encode for the minor pilins of Neisseria gonorrhoeae, with engineered SPI cleavage sites between the hydrophobic inner membrane domain and the soluble headgroup.

Results

Engineered minor pilins were successfully expressed recombinantly in E. coli. These sites proteins are efficiently expressed and cleaved by SPI, separating the soluble domain from the insoluble transmembrane domain. The soluble protein can be extracted from the bacterial periplasm via osmotic shock.

Conclusions

Convenient purification of the soluble domain of minor pilins opens new avenues for exploring the structure and function of the type IV pilus. This could provide new insight into Neisseria pathogenesis.