Analysis of gate residues in the type 2 secretin PulD

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Insanity would have taken me much sooner…

Abstract

Secretins are gated outer-membrane channels with large internal pore sizes (6-10 nm). They are the outer membrane components of bacterial trans-envelope complexes that assemble/export filamentous bacteriophages as well as pili, complex protein toxins and virulence factors. 12-14 identical subunits form the radially symmetrical channels which share a common architecture - a 3-tiered barrel with middle septum. Secretins are essential components of Gram-negative Type 2/3 secretion systems, spanning the outer membrane and interacting with the inner membrane components of transport machinery. Since secretins have such large pore diameters a simple channel would allow noxious compounds through the normally impermeable outer membrane. The presence of a gate structure allows for the controlled opening and closing of secretin channels, in response to specific cues regulating protein export. Here I have determined gate-structural elements of the *Klebsiella oxytoca* Type 2 Secretin, PulD. Random mutagenesis coupled with selection for open or 'leaky'-gate phenotypes created a library of mutations which were mapped by DNA sequence analysis. Analysis of leaky mutants revealed 12 distinct missense point mutations in *pulD*. Additionally, two deletion mutants were isolated, spanning 5 and 9 amino acids, both conferring a leaky gate phenotype. Comparison of these *pulD* mutations with those previously identified in another secretin gene encoding the *Escherichia coli* filamentous phage f1 secretin pIV, reveals mutations in both are localised in two main clusters that correspond to regions within the secretin homology domain. Named GATE1 and GATE2, these clusters indicate functional gate regions in both secretins.

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