Characterization of the secretins, large outer membrane channels of Gram-negative bacteria

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Dedicated to my mother

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"In the name of Allah, the Most Gracious, the Most Merciful"

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Abstract

Secretins, a family of large outer membrane channels, mediate secretion and/or assembly of virulence factors and/or complex proteinaceous structures, such as rods, type IV pili and filamentous bacteriophage. Secretins form large radially-symmetric channels composed of 12 to 14 identical subunits, with internal diameters of up to 10 nm, whose lumen is interrupted by a septum/plug structure that very likely represents a gate or a valve. The identity of septum in the primary sequence of secretins has not been determined as yet, however the cryo-EM and SPA analyses point to the C-terminal domain forming these structures, whereas mutagenesis specifically identified two regions in this domain, named GATE1 and GATE2, as having an important role in gating of the filamentous phage secretion system secretin pIV. However, it is not known whether these regions are also involved in gating of the secretins from type II and type III secretion systems.

In this work, twelve "leaky-gate" mutants in the secretin PulD from the type II secretion system (T2SS), selected from a random mutant library based on ability to utilise 829 Da oligosaccharide maltopentaose in the absence of maltoporin, were analysed in detail. Most of PulD leaky-gate mutants clustered in the GATE1 and GATE2 regions. All point mutants were positive for secretion of the cognate PulD substrate, enzyme Pullulanase (PulA), whereas a 5-residue in-frame deletion (Δ477-481) was negative. Two severely leaky GATE1 region mutants, G458S and Δ477-481, sensitised *E. coli* to all tested antibiotics whose molecular weight is too high to pass through porins: rifamycin SV (720 Da), bacitracin (1423 Da) vancomycin (1449 Da) and daptomycin (1621 Da). The GATE1 of this secretin is therefore a potential drug target, for design of molecules that can sensitise secretin-containing pathogenic Gram-negative bacteria to > 600 Da antibiotics and/or block the secretion of substrates, including virulence factors.

Engineered chimeras between PulD and pIV were used to probe functional compartmentalisation among the secretin domains and the segments involved in gating. This analysis showed that the N-terminal domains, GATE1 region and channel-forming secretin homology domain are interdependent with respect to function in secretion/assembly of the substrates, and to different extends for folding and multimerisation.

This work further analysed the gating properties of the type III secretion system (T3SS) secretins EscC and InvG. When expressed in *E. coli* K12, these secretins were naturally "leaky" and mistargeted to the inner membrane, resulting in growth retardation. The survival of *E. coli* expressing these secretins depended on the PspF, positive regulator of the key inner membrane stress response Psp. Therefore, in the T3SS secretin-expressing or toxin-secreting cells, PspF is a potential target for design of molecules that could kill T3SS-containing toxin-secreting Gram-negative bacteria.

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ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ARFs	ADP ribosylation factors
ATPase	Adenosine triphosphatase
Bac	Bacitracin
Bae	Bacterial adaptive response
Bp	Base pairs
cAMP	Cyclic AMP
CDS	Coding sequence
CFU	Colony forming unit
Cm	Chloramphenicol
Cpx	Conjugative pilus expression response
Cryo-EM	Cryo Electron Microscopy
CtxAB	Cholera toxin AB
Da	Dalton
DNA	Deoxyribonuclic acid
DNS	3,5-dinitrosalicyclic acid
DOC	Deoxycholate
EHEC	Enterohaemorrhagic *Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EOP	Efficiency of plating
EPEC	Enteropathogenic *Escherichia coli*
ESRs	Envelope stress responses
ETEC	Enterotoxigenic *Escherichia coli*
FPSS	Filamentous phage secretion system
GST	Glutathione S-transferase
IPTG	Isopropyl β-D-1-thiogalactopyranoside
Kn	Kanamycin
LEE	Locus of enterocyte effacement
MIC	Minimal inhibitory concentration
MW	Molecular weight

OD	Optical density
PCR	Polymerase chain reaction
Pfu	Plaque forming unit
Pi	Inorganic phosphate
PKA	Protein kinase A
Psp	Phage shock protein response
R	Resistant
Rcs	Regulation of capsular synthesis
RF	Replicative form
RpoE	RNA polymerase, extracytoplasmic stress sigma factor
S	Sensitive
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide
SecYEG	General secretory translocon
SPA	Single particle analysis
SPI	Salmonella pathogenicity island
spp.	species
sv.	serovar
T2SS	Type II secretion system
T3SS	Type III secretion system
T4PBS	Type IV pilus biogenesis system
Tat	Twin-arginine translocon
TCA	Trichloroacetic acid
TEM	Transmission electron microscope
Tet	Tetracycline
Van	Vancomycin
WT	Wild type
Ysa-PI	Yersinia pathogenicity islands