Analysis of *Shigella flexneri* Cell Surface Virulence Factors

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Abstract

The IcsA autotransporter (AT) is a key virulence protein of *Shigella flexneri*, a human pathogen that causes bacillary dysentery through invasion of colonic epithelium. IcsA is a polarly distributed, outer membrane protein that confers motility to intracellular bacteria by engaging the host actin regulatory protein, neural Wiskott-Aldrich syndrome protein (N-WASP). The activated N-WASP in turn activates Arp2/3 complex, which initiates *de novo* actin nucleation and polymerisation to form F-actin comet tails and allow actin-based motility (ABM). The N-terminal surface-exposed IcsA passenger α-domain (aa 53-758) is responsible for N-WASP interaction, where multiple IcsA regions: aa 185-312 (N-WASP interacting region [IR] I), aa 330-382 (N-WASP IR II) and aa 508-730 (N-WASP IR III), have been suggested to be interacting with N-WASP from previous linker-insertion mutagenesis ($IcsA_i$). A putative autochaperone (AC) region (aa 634-735) located at the C-terminal end of IcsA passenger domain, which forms part of the self-associating AT (SAAT) domain, has been suggested to be required for IcsA biogenesis. $IcsA_i$ proteins with linker insertion mutations within the AC region had a significant reduction in production when expressed in smooth lipopolysaccharide (S-LPS) *S. flexneri*.

This thesis investigated the biogenesis of IcsA, seeking to identify factors that affect IcsA AC mutant production in the S-LPS background. $IcsA_i$ AC mutant production was restored to a wild-type comparable levels in the rough LPS (R-LPS) *S. flexneri* (that lack the O-antigen component). The same phenotypes were observed in *S. flexneri* (both S-LPS and R-LPS) expressing site-directed mutagenised IcsA AC protein (aa 716-717). Various approaches were performed to identify the factors that caused different IcsA AC mutant production between S-LPS and R-LPS *S. flexneri.* Both LPS Oag and DegP (a periplasmic chaperone/protease) were identified to affect IcsA AC mutant production in S-LPS strain, as the IcsA AC mutant production was restored in *S. flexneri* Δ*degP* S-LPS strain. In addition, site-directed mutagenesis of residues Y716 and D717 within the AC region showed that these residues are critical for IcsA production and/or stability in the S-LPS background but not in the R-LPS background.

Another aim of this work was to further define N-WASP IRs II and III via site-directed mutagenesis of specific amino acids. Mutant IcsA protein production level, N-WASP recruitment and F-actin comet tail formation by S-LPS and R-LPS *S. flexneri* were characterised. Residues 330-331, and residue 382 within N-WASP IR II, and residues 716-717 within N-WASP IR III, were identified to be involved in N-WASP recruitment. It was shown for the first time that N-WASP activation involves interaction with different regions on different IcsA molecules and hence that oligomeric IcsA is needed for this interaction.

Various "GFP-N-WASP" sub-domain proteins and IcsAα protein were over-expressed, purified and used in protein binding assays. These provided preliminary data for protein binding assays which investigate the relationship between N-WASP and IcsA.

Another *S. flexneri* virulence protein, IcsB, which is involved in preventing autophagy activation by intracellular bacteria was investigated. An *S. flexneri* 2457T Δ*icsB* mutant was created and characterised in this study. In this background, the *icsB* mutation had a less of an effect on plaque formation than reported for another *S. flexneri* background.

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Abbreviations

Abbreviations acceptable to the American Society from Microbiology are used without dentition in this thesis. Additional and frequently used abbreviations are defined when first used in the text, and are listed below.

Å	Angstroms
aa	Amino acids
ABM	Actin-based motility
AC	Autochaperone
Ap	Ampicillin
Ap^R	Ampicillin resistance
Arp	Actin-related protein
AT	Autotransporters
ATP	Adenosine triphosphate
bp	Base pairs
BAM	Beta-barrel assembly machinery
β-ME	β-mercaptoethanol
Cm	Chloramphenicol
Cm^R	Chloramphenicol resistance
CV	Column volume
DAPI	4’,6’-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
DMEM	Dulbecco’s modified Eagle medium
dNTP	Deoxynucleoside triphosphate
DSP	Dithio-bis(succinimidylpropionate)
F-actin	Filamentous actin
FCS	Foetal calf serum
g	Gravitational units
G-actin	Globular actin
GFP	Green fluorescent protein

GRR	Glycine rich repeat
h	Hour(s)
HMW	High molecular weight
$IcsA_i$	IcsA linker-insertion mutant
$IcsA_\Delta$	IcsA deletion mutant
IcsAα::BIO	IcsA with a BIO tag sequence inserted at aa 87
IF	Immunofluorescence
IL	Interleukin
IM	Inner membrane
IPTG	Isopropyl-β-D-thiogalactopyranoside
kb	Kilobases
kDa	Kilodaltons
Km	Kanamycin
Km^R	Kanamycin resistance
L	Litre
LB	Luria Bertani medium
LPS	Lipopolysaccharide
M	Molar
m	mili
M cells	Membranous epithelial cells
min	Minutes
mRNA	Messenger ribonucleic acid
NEB	New England Biolabs
nt	Nucleotide
N-WASP	Neural Wiskott-Aldrich sundrome protein
Oag	O-antigen
OM	Outer membrane
OMP	Outer membrane protein
O/N	Overnight
PAGE	Polyacrylamide electrophoresis

PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PMN	Polymorphonuclear
R-LPS	Rough lipopolysaccharide
rpm	Revolutions per minute
RT	Room temperature
RUs	(Oag) Repeat units
SAAT	Self-associating autotransporter
SAP	Shrimp alkaline phosphatase
sec	Second
SD	Standard deviation
SDS	Sodium dodecylsulphate
S-LPS	Smooth lipopolysaccharide
Sm	Streptomycin
ss	Signal sequence
S-type	Short Oag modal length
Tc	Tetracycline
Tc^R	Tetracycline resistance
Tp	trimethoprim
Tp^R	trimethoprim
TCA	Trichloroacetic acid
TTSS	Type three secretion system
VL-type	Very long Oag modal length
WASP	Wiskott-Alrich syndrome protein
WT	Wild-type
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside
Δ	deletion

Contents