Molecular Characterisation of the Polyhistidine Triad Proteins of *Streptococcus pneumoniae*

THE UNIVERSITY of ADELAIDE

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Abstract

The polyhistidine triad (Pht) proteins are a family of proteins defined by the presence of multiple copies of the histidine triad motif (HxxHxH). There are four members of this family in *Streptococcus pneumoniae*: PhtA, B, D and E. The proteins are found on the cell surface and immunisation with them has been shown to elicit protective immunity against disease caused by this Gram positive pathogen.

The aim of the work presented in this thesis was to extend our understanding of the structure and functions of these proteins, as well as to explore their potential use in vaccines. Firstly, the previously reported interaction of the Pht proteins with factor H (a negative regulator of the alternative pathway of the complement system) was investigated by testing binding of the proteins to different regions of factor H by ELISA and flow cytometry. This revealed that the Pht proteins bind to the first seven domains of factor H more strongly than they do to the full length protein.

Pht proteins have also been implicated in binding to zinc ions. In this work the proteins were found to interact to a certain extent with a number of transition metal ions. However, measurements of metal ion content of wild-type and Δ*phtABDE* mutant strains only showed decreases in zinc and nickel content of the mutant relative to the wild-type. Growth of the mutant strain was impaired relative to wild-type in media with low concentrations of available zinc. Further work indicated that this phenotype is linked to the zinc-specific ABC transporter substrate binding proteins AdcA and AdcAII, and implied that the Pht proteins may facilitate acquisition of zinc by AdcAII.

It is not clear which region or regions of the Pht proteins are required for protective immunity to be induced against pneumococcal disease when used as immunogens. To investigate this, truncated derivatives of PhtA and PhtD were cloned, expressed and purified and analysed for their capacity to bind antibodies that had been generated against the full length protein. This led to the identification of immunogenic regions in both proteins which were subsequently tested as immunogens in mouse models of pneumococcal disease and colonisation. However, significant protective effects were not found in almost all cases, including for control groups immunised with the full length proteins, leading to the conclusion that PhtA and PhtD are not effective vaccine candidates in the models tested.

Lastly, the mechanism of attachment of PhtD to the cell surface was examined by deletion of regions near the N-terminus of the protein and subsequent analysis of the surface accessibilities of the mutant forms of the protein. These experiments identified a short stretch of amino acids that are required for the protein to be cell-associated. Furthermore, a considerable proportion of the total amount of wild-type PhtD produced was found to be released into culture supernatants. Further experiments revealed that the released protein could not re-attach to the surface and that PhtD release occurs in a number of different pneumococcal strains.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Charles Deveron Plumptre and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Abbreviations

°C	degrees Celsius
µg	microgram/s
µl	microlitre/s
µM	micromolar
ABC transporter	ATP-binding cassette transporter
ATP	adenosine triphosphate
bp	base pairs
C+Y	casein hydrolysate medium with yeast extract
CFU	colony forming units
cml	chloramphenicol
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid disodium salt
ELISA	enzyme-linked immunosorbent assay
ery	erythromycin
FITC	fluorescein-5-isothiocyanate
g	relative centrifugal force
h	hour/s
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid
ICPMS	inductively-coupled plasma mass spectrometry
IPTG	isopropyl-β-D-thio-galactopyranoside
kb	kilobase/s
K_D	dissociation constant
kDa	kilodalton/s
kg	kilogram/s
kpsi	kilopounds per square inch
l	litre/s
LB	Luria Bertani broth
M	molar
mg	milligram/s
min	minute/s

ml	millilitre/s
mM	millimolar
MOPS	3-(N-morpholino)propanesulphonic acid
ng	nanogram/s
Ni-NTA	nickel-nitrilotriacetic acid
nm	nanometres
OD_{600}	optical density at 600 nm
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PMSF	phenylmethylsulphonyl fluoride
RT	room temperature
s	second/s
SBP	solute-binding protein
SDS	sodium dodecyl sulphate
spec	spectinomycin
spp	species
TBE	tris-borate EDTA buffer
tet	tetracycline
TPEN	N,N,N′,N′-Tetrakis(2-pyridylmethyl)ethylenediamine
TTBS	Tween tris buffered saline
v/v	volume per volume
w/v	weight per volume
WT	wild-type

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