The Molecular Mechanisms of Metal Ion Homeostasis in *Streptococcus pneumoniae*

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Table of Contents

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Thesis Style and Layout

The contents of this thesis have been presented as both published and unpublished works. Chapter 1 'Introduction' presents the current literature and frames the research aims and questions of my doctoral studies. Chapter 2 'Materials & Methods' contains only the methods that have not been explicitly detailed in the individual chapters due to space restrictions imposed by the respective journals. All oligonucleotides generated for this study can be found in their respective chapters. Chapters 3, 4 and 5 are published, peer-reviewed, research articles produced during my candidature. These publications have been included as they were published, leading to slight differences in nomenclature and language (i.e. Australian vs American spelling). Each publication is preceded by a statement outlining the purpose of the study and the research aims addressed by the work. Authorship statements denoting the contribution of each of the authors have also been included for each article, and have been verified and signed by all co-authors. Published versions of the articles have been included in Chapter 8 'Appendices' with permission from the copyright holder. Chapter 6 contains currently unpublished data and has therefore been written up as a conventional thesis chapter. Chapter 7 'Final Discussion' discusses the findings presented in this thesis, highlighting the implications of this work for the field of bacterial cellular chemistry, and suggests future directions for this area of study. A complete reference list, including references from both published and unpublished chapters has been included in Chapter 9.

Abstract

All living organisms have an absolute requirement for transition metal ions. In the human pathogen *Streptococcus pneumoniae,* preservation of homeostatic metal ion concentrations is crucial for the viability and virulence of the organism. As a strictly host-adapted pathogen, *S. pneumoniae* is beholden to the prevailing metal abundance of the host environment, and must selectively acquire poorly-abundant essential metals, while also preventing toxicity from metal ions present in excess. This requires an intricate network of transcriptional regulators and transport pathways, which rapidly respond to changes in the extracellular metal ion composition. However, the molecular details underlying aspects of this process, such as how metal binding proteins achieve ligand specificity, remain poorly understood. Furthermore, the consequences of loss or failure of these mechanisms, such as the binding of incorrect metals to proteins (mismetallation), have not been thoroughly investigated.

Here, we have examined the maintenance of metal ion homeostasis, first at the molecular level, with an investigation of PsaA, the only high affinity manganese-acquisition protein in *S. pneumoniae*. Acquisition of manganese is crucial for viability and virulence of the pneumococcus in the host. However, the mechanisms used by PsaA to achieve selectivity for manganese, while excluding more competitive metals such as zinc, were unknown. Through molecular characterisation of PsaA, this study identified a novel mechanism of metal binding and release, providing an explanation for the hitherto unexplained metal-binding behaviour of PsaA seen in previous studies. Additionally, the limit of chemical-selectivity for PsaA was investigated through the use of cadmium, a non-physiological metal, which was able to subvert the selectivity determinants of PsaA, leading to cellular cadmium toxicity.

Investigation of cadmium toxicity revealed that the phenotypic and cellular consequences of toxicity arose primarily from dysregulation of the manganese and zinc homeostatic mechanisms, resulting from cadmium mismetallation of cellular metalloproteins. The extent of cadmium mismetallation was investigated through a combination of transcriptomics, metabolomics, and metalloproteomics, revealing that cadmium could potentially mismetallate numerous metal-dependent proteins, resulting in altered gene expression and metabolomic profiles, due to changes in carbon source utilisation, cellular energy production and fatty acid biosynthesis pathways.

Collectively, these investigations allowed for the proposal of a novel molecular mechanism of cadmium toxicity in *S. pneumoniae* and provided new insights into the intracellular networks that facilitate bacterial metal ion homeostasis. The structural and mechanistic analyses

of PsaA have shown how metal ion discrimination could be achieved *in vivo*, and provided high-resolution data for the rational design of novel antimicrobial therapeutics to selectively target this highly-conserved virulence determinant.

Publications

Peer Reviewed Primary Research Articles

Begg, S.L., Eijkelkamp, B.A., Luo, Z., Couñago, R.M., Morey, J.R., Maher, M.J., Ong, C.Y., McEwan, A.G., Kobe, B., O'Mara, M.L., Paton J.C. and McDevitt, C.A. (2015) Dysregulation of transition metal ion homeostasis is the molecular basis for cadmium toxicity in *Streptococcus pneumoniae. Nature Communications*, 6, Article No: 6418

Deplazes, E.*, **Begg, S.L.***, van Wonderen, J.H.*, Campbell, R., Kobe, B., Paton, J.C., MacMillan, F., McDevitt, C.A. and O'Mara, M.L. (2015) Characterizing the conformational dynamics of metal-free PsaA using molecular dynamics simulations and electron paramagnetic resonance spectroscopy. *Biophysical Chemistry*, 207:51-60
*Equally contributing first authors

Bajaj, M., Mamidyala, S.K., Zuegg, J., **Begg, S.L**., Ween, M.P., Huang, J.X., Kobe, B., Paton, J.C., McDevitt, C.A. and Cooper, M. (2015) Discovery of Novel Pneumococcal Surface Antigen A (PsaA) Inhibitors Using a Fragment-based Drug Design Approach. *ACS Chemical Biology*, 10 (6): 1511-1520

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Couñago, R.M.*, Ween, M.P.*, **Begg, S.L**., Bajaj, M., Zuegg, J., O'Mara, M.L., Cooper, M.A., McEwan, A.G., Paton, J.C., Kobe, B. and McDevitt, C. A. (2014) Imperfect coordination chemistry facilitates metal ion release in the Psa permease. *Nature Chemical Biology*, 10 (1): 35-41
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Pederick, V.G., Eijkelkamp, B.A., Ween, M.P., **Begg, S.L**., Paton, J.C. and McDevitt, C.A. (2014) Acquisition and Role of Molybdate in *Pseudomonas aeruginosa. Applied and Environmental Microbiology*, 80 (21): 6843-6852

Book Chapters

McDevitt, C.A., **Begg, S.L**. and Paton, J.C. (2015) Metal ion toxicity and oxidative stress in *Streptococcus pneumoniae*. In "Stress and Environmental Control of Gene Expression in Bacteria" 1184-1193 Editor F.J. de Bruijn, Publisher John Wiley & Sons, Inc.

Abbreviations

A	Absorbance
ABC	ATP binding cassette
ACN	Acetonitrile
AEX	Anion exchange chromatography
AGRF	Australian genome research facility
Ala	Alanine
Asp	Aspartate
ATP	Adenosine triphosphate
BA	Blood agar
bp	Base pairs
Ca	Calcium
Cd	Cadmium
CDF	Cation diffusion facilitator
CDM	Cation defined media
CFU	Colony forming units
Co	Cobalt
CPS	Capsular polysaccharide
c.p.s	Counts per second
CSP	Competence stimulating peptide
Cu	Copper
cw	Continuous wave
Cys	Cysteine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EPR	Electron paramagnetic resonance
Ery	Erythromycin
Ery^R	Erythromycin resistance cassette
FA	Fatty acid
Fe	Iron
fL	femtolitre
g	Relative centrifugal force
gDNA	Genomic DNA
Glu	Glutamate
GROMACS	Groningen Machine for Chemical Simulation
GSH	Glutathione
h	Hours
His	Histidine
HPLC	High pressure liquid chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
ID	Identification
Ile	Isoleucine
IPD	Invasive pneumococcal disease

K	Degree Kelvin
Kan	Kanamycin
Kan^R	Kanamycin resistance cassette
K_D	Dissociation constant
kDa	Kilodalton
kJ	Kilojoule
kPSI	Knots per square inch
L	Litre
LC-ICP-MS	Liquid chromatography inductively coupled plasms mass spectrometry
LB	Luria Bertani (lennox) broth
Leu	Leucine
LIC	Ligation independent cloning
LPS	Lipopolysaccharide
Lys	Lysine
M	Molar
MD	Molecular dynamics
MES	2-(*N*-morpholino)ethanesulfulfonic acid
Mg	Magnesium
mg	Milligrams
min	Minutes
mL	Millilitres
mM	Millimolar
Mo	Molybdenum
Mn	Manganese
MOPS	3-(*N*-morpholino)propanesulfonic acid
MS	Mass spectrometry
MTSL	*S*-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonothioate
n	Number of replicates
NBD	Nucleotide binding domain
NCBI	National Centre for Biotechnology Information
n.d.	Not detected
ng	Nanogram
Ni	Nickel
nM	Nanomolar
nm	Nanometers
NRAMP	Natural resistance-associated macrophage protein
n.s.	Not significant
ns	Nanosecond
OD	Optical density
P	Phosphorous
PBP	Penicillin binding protein
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine
PDB	Protein data bank

PEG	Polyethylene glycol
PIPES	Piperazine-N,N'-bis(2-ethanesulfonic acid)
pm	picometres
ppb	Parts per billion
ps	picoseconds
qRT-PCR	Quantitative reverse transcription PCR
ROS	Reactive oxygen species
RMSD	Root mean square deviation
RMSF	Root mean square fluctuation
RNA	Ribonucleic acid
RND	Resistance nodulation division
RNS	Reactive nitrogen species
rRNA	Ribosomal RNA
s.e.m.	Standard error of the mean
s.d.	Standard deviation
SDM	Site-directed mutagenesis
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
sec	Seconds
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
SBP	Substrate binding protein
Str	Streptomycin
Str^R	Streptomycin resistance cassette
TAE	Tris-HCl, acetic acid and EDTA buffer
TB	Terrific broth media
TC	Two component
TCEP	Tris(2-carboxyethyl)phosphine
TFA	Trifluoroacetic acid
THY	Todd-Hewitt media + yeast extract
Tm	Melting temperature
TSA	Thermal shift assay
Tyr	Tyrosine
V	Vanadium
Val	Valine
W	Tungsten
WT	Wild-type
Zn	Zinc
°	Degree
°C	Degree Celsius
µg	Microgram
µL	Microlitre
µM	Micromolar
µm	Micrometre
Å	Angstrom

Contributions

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