Molecular Mechanism of Alginate Polymerisation and Modifications in *Pseudomonas aeruginosa*

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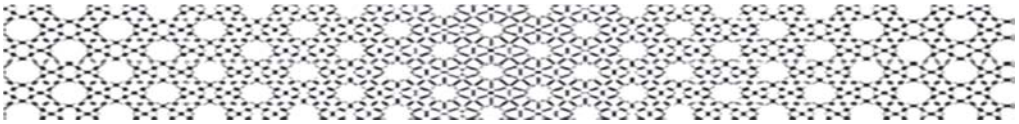
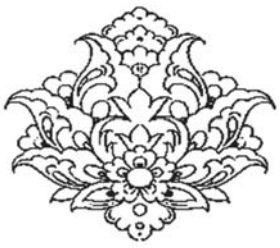


In Loving Memory of My Mom

Pari



"Science is a way of thinking much more than it is a body of knowledge"

Carl Sagan

Abstract

Pseudomonas aeruginosa is an ubiquitous opportunistic human pathogen in immunocompromised patients. It is of particular relevance to cystic fibrosis (CF) patients where it frequently causes chronic bronchopulmonary infection and is the leading cause of morbidity and mortality. The decline in lung function is caused by the emergence of a mucoid variant showing excessive production of the exopolysaccharide, alginate. The alginate-containing biofilm matrix of this mucoid variant protects *P. aeruginosa* from the immune system and antibiotics.

Here the alginate biosynthesis/modification/secretion multiprotein complex was investigated with regard to protein-protein interactions constituting the proposed multiprotein complex and the molecular mechanisms underlying alginate polymerisation and modifications. This study sheds light on the structure and function of various alginates from a material property and biological function perspectives. The binary interactions of AlgK-AlgE, AlgX-Alg44, AlgK-Alg44 and Alg8-Alg44 were identified proposing a new model for this multiprotein complex organisation. Protein-protein interactions were found to be independent of c-di-GMP binding to $PilZ_{Alg44}$ domain. C-di-GMP-mediated activation of alginate polymerisation was found to be different from activation mechanism proposed for cellulose synthesis. This study showed that alginate polymerisation and modifications were linked. It was shown that the molecular mass of alginate was reduced by epimerisation, while it was increased by acetylation. It was determined that previously characterized proteins AlgG (epimerase) and AlgX (acetyltransferase) have mutual auxiliary and enhancing roles. Biofilm architecture analysis showed that acetyl groups lowered viscoelasticity of alginates and promoted cell aggregation, while nonacetylated polymannuronate alginate promoted stigmergy. Experimental evidence was provided that Alg44 boosted acetylation while the periplasmic domain of this protein was critical for protein stability and regulation of alginate modifications. Full-length Alg44 was purified and it was found to be a dimer in solution. Overall, this study sheds new light on the arrangement of the proposed alginate biosynthesis/modification/secretion multiprotein complex. Furthermore, the activation mechanism and the interplay between polymerisation and modification of alginate were elucidated and new functions and interactive role of alginate-polymerising and–modifying subunits were further understood.

Preface

The format of this thesis complies with the "submission of thesis based on publications" as described in the latest version of the Handbook for Doctoral studies, version 7, published by the Massy University doctoral research committee (January 2011).
The following sections of this thesis have been published or submitted or drafted for publication in internationally peer-reviewed journals. Publications have not been arranged in chronological order.

Chapter II

Hay ID, Ur Rehman Z, Moradali MF, Wang Y, Rehm BHA (2013). Microbial Alginate Production, Modification and its Applications. Microbial Biotechnology **6:** 637–650.

Chapter III

Hay ID, Wang Y, Moradali MF, Ur Rehman Z, Rehm BHA (2014). Genetics and Regulation of Bacterial Alginate Production. Environmental Microbiology **16:** 2997-3011.

Chapter IV

Rehman ZU, Wang Y, Moradali MF, Hay ID, Rehm BHA (2013). Insight into Assembly of the Alginate Biosynthesis Machinery in *Pseudomonas aeruginosa*. Applied and Environmental Microbiology **79:** 3264-3272

Chapter V

Moradali MF, Donati I, Sims IM, Ghods S, Rehm BHA (2015). Alginate Polymerisation and Modification are Linked in *Pseudomonas aeruginosa*. (mBio 6(3):e00453-15)

Chapter VI

Moradali MF, Rehm BHA (2015). The Role of Alg44 in Alginate Synthesis and Modification (Draft manuscript)

Contributions Moradali MF made to publications are as follows

Chapter II: This review was drafted by I.D.H, Z.U.R, M.F.M and W.Y, and finalised by B.H.A.R

Chapter III: This review was drafted by I.D.H, W.Y, M.F.M and Z.U.R and finalised by B.H.A.R

Chapter IV: Chromosomal integration of all the genes was designed and made by Z.U.R. Generation of PDO300Δ*algK* knock-out and its complementation was done by Y.W. AlgE co-immunoprecipitation was done by Z.U.R. AlgK pull-down was done by Y.W and PDO300 Δ*alg44*Δ*algX* mutant was created by I.D.H. This mutant was re-confirmed and transformed with pBBR1MCS-5:*alg44-6his* by M.F.M. *In vivo* chemical cross-linking and Alg44 pull-down assay was performed by M.F.M. Manuscript was drafted by Z.U.R, Y.W and M.F.M and I.D.H and finalised by B.H.A.R.

Chapter V: Generation of double-gene knockout mutants in *alg8*/*alg44*, *algG*/*algX* and *algG*/*alg44*, single-gene knockout mutant in *algG* and re-confirming single-gene knockout mutants in *alg8*, *alg44 and algX*, construction of plasmids for complementing these mutants, chromosomal integration of the genes and site-specific mutations and deletions for producing Alg8, Alg44, AlgX and AlgG variants, *in vivo* detection of protein-protein interaction network, isolation of cytoplasmic membrane and general protein analysis and all other assessments were performed by M.F.M. ^{1}H-NMR analysis was done and interpreted by I. D and M.F.M. SEC-MALLS analysis was performed by I.S and M.F.M. Setting up and analysis of biofilms was performed by M.F.M and S.G. Manuscript was mainly drafted by M.F.M. and finalized by B.H.A.R.

Chapter VI: All of the experimental work was done by M.F.M. Manuscript was drafted by M.F.M and finalised by B.H.A.R.
DNA sequencing was provided by external services.

This is to certify that above mentioned work was conducted by M. Fata Moradali.

Signature	Date	Signature	Date
Prof. Bernd H.A. Rehm		M. Fata Moradali	

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My dear wife Shirin, as a token of appreciation for your love, your loyalty, your support and tireless efforts on my behalf, I want to publicly tell you: Thank You.

Also, many thanks to Prof. Hedjaroude, my previous supervisor, who changed my life by encouraging and supporting me in the past and present, and thanks to my family-in-law for their supports. Many thanks to my parents especially to my Mom who is now watching over me with love from heaven; I do it in honour of you, dear Pari. Finally many thanks to Massey University and IFS for all the support.

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LIST OF ABBREVIATIONS

°C Degree Celsius
Ap Ampicillin
BSA Bovine serum albumin
Cb Carbenicillin
Δ Delta (deleted)
DMSO Dimethyl sulfoxide
D_2O Deuterium oxide
DNA Deoxyribonucleic acid
DNAase Deoxyribonuclease
dNTPs Deoxyribonucleotide triphosphates
EtOH Ethanol
EDTA Ethylenediaminetetraacetic acid
g gravity/gram
Gm gentamycin
GTP Guanosine triphosphate
HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRP Horse radish peroxidase
IPTG Isopropyl â-D-1-thiogalactopyranoside
kDa Kilo Daltons
λ Lambda (wavelength or type of phage)
LB Luria-Bertani (broth)
OD Optical density
ORF Open reading frame
PCR Polymerase chain reaction
PIA Pseudomonas isolation agar
PPI Protein-protein interaction
RNAase Ribonuclease
SD Standard deviation
SDS-PAGE Sodium dodecyl sulfate gel electrophoresis
TBE Tris-Borate-EDTA buffer viii
Tc Tetracycline
TE Tris-EDTA buffer
Tm Primer melting temperature
Tris Trishydroxymethylaminomethane
vol/vol Volume per volume
wt/vol Weight per volume
X-Gal 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside