Molecular characterisation of PHA synthase and the *in vivo* synthesis of functionalised PHA beads with surface immobilised proteins

A thesis presented in partial fulfilment of the
requirements of the degree of
Master of Science
in
Microbiology
at Massey University, Palmerston North,
New Zealand.

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2011

Abstract

Polyhydroxyalkanoates (PHAs) are naturally occurring biopolyesters, synthesized by a large range of bacteria and deposited as small spherical water-insoluble cytoplasmic inclusion bodies containing hydrophobic polyester core surrounded by a phospholipid monolayer and associated embedded proteins. The most common form of PHA identified in bacteria is polyhydroxybutyrate (PHB).

Formation of PHA beads requires three important enzymes with PHA synthase (PhaC) being the most important, catalysing the final stereo-selective conversion of (*R*)-3-hydroxyacyl-CoA thioesters into PHA. Increasingly beads are used as microbeads, which display surface immobilised proteins for a range of applications in biotechnology and medicine.

However, functionalised PHA beads are largely produced in Gram-negative bacteria which contain endotoxins that are known to co-purify with the beads and are considered undesirable in medical applications. In addition, despite extensive research towards understanding PHA synthases, to date no structural data is currently available.

Here it was shown that functionalised PHB beads can be produced *in vivo* for both the purification of antibodies and the display of medically relevant antigens (e.g. Hepatitis C) on the surface of PHB beads from the Gram-positive bacterium *L.lactis*. In addition, it was shown that PHA synthase from *R.eutropha* can be highly overproduced, remains largely soluble and can be purified to greater than 90 % purity.

The results demonstrated and supported the use of PHB beads as a platform for the production of functionalised PHA beads suitable for a large range of biotechnological or medical applications. Although no structural data for PHA synthases are currently available, our results demonstrate progress towards obtaining a three-dimensional protein structure for PHA synthase (PhaC).

Acknowledgement

"Success, 100 % persistence and a bit of luck"

I would like to first of all give special thanks to my supervisor Professor Bernd Rehm for allowing me the opportunity to do my Masters qualification under his supervision. I would also like to thank Bernd and a special mention to Zoe Jordens for their time, guidance and encouragement during my postgrad years.

Special thanks goes out to Jane Mullaney for her encouragement, support and technical know how at the start of my postgrad year. Also, special thanks to Anika Jahns and Ian Hay for their endless expertise and help when required. In addition, special thanks to Andrew Sutherland-Smith, Greg Sawyer and everyone else in the protein engineering lab for their time, advice, and help with things protein related.

Thanks to my all my colleges in the Rehm lab, Paul Blatchford for all those coffee breaks and to everyone at IMBS.

This would not have been possible if it wasn't for Tracy Thompson and Polybactics who provided financial assistance during my final year of postgrad.

And last but not least, thanks to my wonderful girlfriend Yifang Tay for her endless encouragement and support.

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Abbreviations

°C	Degree Celsius
$3HA_{MCL}$	Medium chain length (*R*)-3-hydroxy fatty acids
$3HA_{SCL}$	Short chain length (*R*)-3-hydroxy fatty acids
3HB	3-hydroxybutyrate
AGE	Agarose Gel Electrophoresis
BSA	Bovine serum albumin
DMSO	Dimethyl sulfoxide
FM	Fluorescent Microscopy
GAP	Bead Associated Proteins
GC/MS	Gas chromatography mass spectrometry
HRP	Horse radish peroxidise
IgG	Immunoglobulin G
IMAC	Immobilised Metal Affinity Chromatography
kDa	Kilo Daltons
LDH	Lactate dehydrogenase
Maldi-TOF\MS	Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry
PBS	Phosphate buffered saline
PHA	Polyhydroxyalkanoate
phaCAB	PHA operon
PhaC	PHA synthase
PhaE	Type II PHA synthase subunit
PhaP	Phasin regulatory protein
PhaZ	PHA intracellular depolymerase
PHB	Polyhydroxybutyrate
RBS	Ribosome binding site
RE	Restriction endonuclease
SDS-PAGE	Sodium dodecyl sulfate gel electrophoresis
TEM	Transmission Electron Microscopy
TEV	Tobacco Etch Virus protease
WT	Wildtype

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