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Polyester synthases and polyester granule assembly

A thesis presented to Massey University in partial fulfilment of the requirement for the degree of Doctor of Philosophy in Microbiology

Verena Peters

2008

Dedicated to my parents

Meinen Eltern in Dankbarkeit gewidmet

The most exciting phrase to hear in science, the one that heralds new discoveries, is not “Eureka!” (“I found it!”), but rather, "hmm.... that's funny...."

Isaac Asimov (1920-1992), biochemist and science fiction author

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Preface

This thesis is written according to the regulations of the latest version of the Handbook for Doctoral Study, published by the Doctoral Research Committee in April 2007 (GRS version 3). The thesis format complies with the format of a thesis based on publications, as described on page 61-62 under chapter "Submission of a thesis based on publications".
Below, all chapters which are published or submitted for publication are listed. These publications do not appear in chronological order.

Chapter I B:

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Chapter II:

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Chapter III:

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Chapter IV:

Jane A. Brockelbank, Verena Peters and Bernd H. A. Rehm (2006). Recombinant *Escherichia coli* produces ZZ domain displaying biopolyester granules suitable for IgG purification.

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Chapter V:

Verena Peters and Bernd H. A. Rehm (2008). Protein engineering of streptavidin for *in vivo* assembly of streptavidin beads.

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Listing of research contributed to the publications/chapters by Verena Peters

Chapter I B: The book chapter was partly written by Verena Peters, with particular emphasis on structure, associated proteins, assembly process and biotechnological potential of PHA granules.

Chapter II: All experiment regarding the polar positional information of the PHA synthase were conducted by Verena Peters, including primer/vector design and cloning, mutagenesis of the PHA synthase, translational fusions to GFP and fluorescent microscopy imaging. The localisation experiments of the phasin protein were also performed by Verena Peters, including primer and vector design and cloning, expression experiments and fluorescent microscopy imaging. Dorit Becher did all in-frame linker/hemagglutinin insertion mutagenesis experiments including the mutant analyses. Verena Peters repeated the preparation of samples for GC/MS analyses of all linker PHA synthase insertion mutants and all hemagglutinin PHA synthase insertion mutants.

Chapter III: All experiments were performed by Verena Peters. Plasmid pBHR80AlacZ was constructed by Verena Peters during her diploma thesis. Jane Brockelbank is acknowledged for her SDS-PAGE analysis of whole cell extract of *Escherichia coli* expressing the fusion protein under lac promoter control. Isogenic marker-free Δ*phaC1-Z*-Δ*C2* deletion mutant of *P. aeruginosa* PAO1 was provided.

Chapter IV: Verena Peters designed all primers/vectors used in this study. The cloning strategy was developed by Verena Peters, and she also conducted the preparative SDS-PAGE analyses of fusion proteins for MALDI-TOF/MS analyses.

Chapter V: All experiments were done by Verena Peters. Natalie Parlene is acknowledged for her assistance in operating the FACS instrument.

A service provider was used for DNA Sequencing, TEM, MALDI-TOF/MS and GC/MS analyses.

This is to certify that the above mentioned research has been conducted by Verena Peters.

(Date, Signature)	(Date, Signature)
Prof. Bernd H. A. Rehm	Verena Peters

Abstract

PHAs are a class of biopolymers consisting of (*R*)-3-hydroxy-fatty acids and are produced by the majority of eubacteria and some archaeal bacteria as carbon storage material. In general, PHA is synthesised when a carbon source is available in excess while another essential nutrient is limited. The key enzyme of PHA biosynthesis, the PHA synthase, catalyses the polymerisation of the substrate (*R*)-3-hydroxyacyl-CoA to PHA accompanied by the release of coenzyme A. PHA is stored intracellularly as inclusions, the so-called PHA granules. When the external carbon source becomes exhausted, bacteria can metabolise these carbon inclusions by degradation of the polymer.

PHA granules are water-insoluble, spherical inclusions of approximately 50-500 nm in diameter which consist of a hydrophobic polyester core surrounded by a phospholipid layer with embedded and attached proteins. One could consider isolated PHA granules as bio-beads due to their structure and size. In this study we tested if the PHA synthase can be used as an anchor molecule in order to display proteins of interest at the PHA granule surface. Furthermore, these modified PHA granules were analysed for their potential applicability as bio-beads in biotechnological procedures. The concept of using the PHA synthase as granule-anchoring molecule for display of proteins of interest was established by the functional display of the β-galactosidase at PHA granules. This "proof of concept" was followed by the display of biotechnologically more interesting proteins. The IgG binding domain of protein A as well as streptavidin, which is known for its biotin binding ability, were fused to the PHA synthase, respectively, and therefore localised at the PHA granule surfaces during PHA granule assembly, resulting in functional bio-protein A -beads and bio-streptavidin-beads. Moreover, their applicability in biotechnological assays was demonstrated.

Recently, we fused the green fluorescent protein (GFP) to the PHA synthase and demonstrated that the PHA granule assembly does not start randomly distributed in the cytoplasm but occurred localised at or near the cell poles. To further investigate if the localisation of the PHA granule formation process is due to polar positional information inherent to the PHA synthase, different mutated versions of the PHA synthase of *Cupriavidus necator* were created and analysed for a potential alteration in localisation. Furthermore, the phasin protein PhaP1 of *C. necator* was fused to HcRed, a far-red fluorescent protein, and localisation studies were accomplished when the fusion protein was expressed under different conditions in *Escherichia coli*.

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Abbreviations

Abbreviation	Meaning
A	Alanine
BChl	Bacteriochlorophyll
C	Cysteine
°C	Degrees Celsius
CoA	Coenzyme A
D	Aspartic acid
GAP	Granule associated protein
GC	Gas chromatography
GFP	Green fluorescent protein
Gm	Gentamycin
H	Histidine
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence activated cell sorting
Fig.	Figure
kDa	Kilo Dalton
LacZ	β-Galactosidase
LB	Luria-Bertani
MALDI-TOF	Matrix-assisted laser desorption/ionisation time-of-flight
MS	Mass spectrometry
Mw	Molecular weight
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PHA	Polyhydroxyalkanoate
PP	Polypropylene
RNA	Ribonucleic acid
Rubisco	Ribulose-1,5-bisphosphate carboxylase/oxygenase
S	Serine
SA	Streptavidin
SDS	Sodium dodecyl sulphate
TEM	Transmission electron microscopy
WS/DGAT	Wax ester synthase / diacylglycerol acyltransferase
(wt/vol) or (w/v)	Weight per volume
(vol/vol) or (v/v)	Volume per volume
YFP	Yellow fluorescent protein
SDS	Sodium dodecyl sulphate