Towards a better understanding of the polyhydroxyalkanoate synthase from *Ralstonia eutropha*: Protein engineering and molecular biomimetics

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With love and gratitude to

Mum & Dad

And in memory of my grandmother

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Preface

This thesis is written according to the regulations of the latest version of the Handbook for Doctoral Studies, version 5, published by the Doctoral Research Committee in January 2009. The format of this thesis complies with the format of a thesis based on publications as described in the chapter "Submission of a thesis based on publications" on page 64.

All chapters that are published or submitted for publication are listed below. These contributions do not appear in chronological order.

Chapter I B

Indira A. Rasiah, Natalie Parlane, Katrin Grage, Rajasekaran Palanisamy, Anika C. Jahns, Jane A. Atwood and Bernd H. A. Rehm (2009). Biopolyester particles: preparation and applications. **Encyclopedia of Industrial Biotechnology**: in press

Chapter I C

Katrin Grage, Anika C. Jahns, Natalie Parlane, Rajasekaran Palanisamy, Indira A. Rasiah, Jane A. Atwood and Bernd H. A. Rehm (2009). Bacterial polyhydroxyalkanoate granules: Biogenesis, structure and potential use as micro-/nano-beads in biotechnological and biomedical applications. **Biomacromolecules 10(4):** 660-669

Chapter II

Anika C. Jahns and Bernd H. A. Rehm (2009). The class I polyhydroxyalkanoate synthase from *Ralstonia eutropha* tolerates translational fusions to its C terminus: A new mode of functional display. **Applied and Environmental Microbiology**: in press

Chapter III

Anika C. Jahns, Richard G. Haverkamp and Bernd H. A. Rehm (2008). Multifunctional Inorganic-Binding Beads Self-Assembled Inside Engineered Bacteria. **Bioconjugate Chemistry 19(10):** 2072-2080

Chapter IV

Anika C. Jahns, Verena Peters and Bernd H. A. Rehm (2009). Engineering of bacterial polyester inclusions towards the display of ten lysine residues and potential applications. **Journal of Biomedicine and Biotechnology** – submitted for publication (2. Submission in revised form)

Listed below are all research contributions to the chapters/publications performed by Anika Jahns:

Chapter I B: The review was partly written by Anika Jahns, focussing on the background description and particle formation of polymalate and the applications of PHA particles.

Chapter I C: The parts of the review article describing the phasins and the regulatory proteins were contributed by Anika Jahns.

Chapter II: All experiments were performed by Anika Jahns. Verena Peters is acknowledged for constructing the plasmid $pCWE_{Spe}$-Mpl-EC.

Chapter III: Except for AFM measurements, all experiments were performed by Anika Jahns. Richard G. Haverkamp obtained all AFM data.

Chapter IV: Verena Peters constructed the plasmids pHAS+phaPwt and pHAS+phaPpolylys. All further experiments regarding the granule isolation, characterization and identification of the respective proteins and the silica binding assays were performed by Anika Jahns.

DNA sequencing, MALDI-TOF/MS, GC/MS and TEM analyses were provided by external services.

This is to certify that the above mentioned research has been conducted by Anika Jahns.

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

(Date, Signature)
Anika Jahns

Abstract

Polyhydroxyalkanoates (PHAs) are polyesters composed of *(R)*-3-hydroxy-fatty acids. A variety of gram-positive as well as gram-negative bacteria and some archaea are able to produce these biopolymers as energy and carbon storage materials. In times of unbalanced growth, when carbon is available in excess but other nutrients are limited, PHA inclusions are formed. These granules are water-insoluble, stored intracellularly and can be maintained outside the cell as beads. The key enzyme for the formation of PHA inclusions is the PHA synthase PhaC, which catalyses the polymerization of *(R)*-3-hydroxyacyl-CoA to PHA with the concomitant release of CoA.

The PHA synthase from *Ralstonia eutropha* (currently *Cupriavidus necator*), which is covalently bound to the PHA granule surface, tolerates fusions to its N terminus without loss of activity. In this study it was investigated if it would also tolerate translational fusions to its C terminus. A specially designed linker was employed, aiming at maintaining the hydrophobic surroundings of the *R. eutropha* synthase C terminus to allow proper folding and activity. Two reporter proteins were tested as fusion partners, the maltose binding protein MalE and the green fluorescent protein GFP. As GFP is a hydrophobic protein itself, no additional linker between the PHA synthase and the reporter protein was necessary to produce PHA granules displaying the functional fusion protein on the surface. Principally, the PHA synthase PhaC tolerates translational fusions to its C terminus but the nature of the fusion partner influences the functionality.

Recently, PHA granules have often been acknowledged as bio-beads. A one-step production allows the formation of functionalised beads without the need for further cross-linking to impart desired surface properties. PHA beads displaying a gold- or silica-binding peptide at the N terminus of PhaC were constructed and tested for their applicability. Additionally, these beads were able to bind IgG due to the ZZ domain of the IgG binding protein A, which was employed as a linker sequence. These functionalised beads can be used as molecular tools in bioimaging and biomedicine, combining organic core with inorganic-binding shell structures.

In a different biomimetic approach, the display of ten lysine residues at the granule surface was achieved using the phasin protein PhaP as the anchoring matrix. Extensive work was performed in an attempt to also employ the synthase protein, but was unsuccessful. These positively charged bio-beads can be used for dispersion or cross-linking experiments as well as silica binding.

Table of Contents

Chapter IV

Chapter V

Chapter VI

List of Figures

List of Tables

Abbreviations

AFM	Atomic Force Microscopy
Au	Gold (aurum)
BSA	Bovine serum albumin
˚C	Degrees Celcius
CoA	Coenzyme A
d	Density
DEAE	Diethylaminoethyl cellulose
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence activated cell sorting
Fig.	Figure
GAP	Granule associated protein
GC	Gas chromatography
GFP	Green fluorescent protein
GTP	Guanosine triphosphate
HPLC	High performance liquid chromatography
IgG	Immunoglobulin G
kDa	Kilo Dalton
LacZ	β-Galactosidase
LB	Luria-Bertani
M	Molar (mol/l)
MALDI-TOF	Matrix-assisted laser desorption ionisation/time-of-flight
MalE	Maltose binding protein
MS	Mass spectrometry
Mw	Molecular weight
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PHA	Polyhydroxyalkanoate
PHB	Poly(3-hydroxybutyrate)
PLA	Polylactide
PLGA	Poly(lactic-co-glycolic acid)
PMLA	Poly(β-L-malate)
SDS	Sodium dodecyl sulphate
TEM	Transmission Electron Microscopy