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# **FUNCTIONAL DISPLAY OF IMMUNOGLOBULIN BINDING DOMAINS ON THE SURFACE OF BIOPOLYMER BEADS**

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## ABSTRACT

Protein A, G and L are immunoglobulin binding proteins isolated from the cell wall of certain gram positive bacteria. The interaction between the binding domain of these proteins and the immunoglobulin molecule occur without affecting the functional Fc region of the antibody, thus making them an ideal tool for antibody purification. The capacity of protein A to bind IgG with such high affinity is the driving motivation for its industrial scale use for immunoglobulin purification such as in chromatography resins. A major disadvantage however, is the inability of protein A to bind certain subclasses of IgG as well as IgG from certain species; a pitfall that can be overcome with the display of either protein G in combination with protein A, or with protein L which binds a range of immunoglobulin based on light chain interactions. Here we display both the binding domain of protein A with the binding domain of protein G on a single platform; the surface of polyhydroxyalkanoate (PHA) biopolyester granules. We also produce a PHA granule displaying the binding domain of protein L. This was achieved via fusion and expression of the genes for these immunoglobulin binding bacterial proteins and the *phaC* gene on a single plasmid construct. The *phaC* gene codes for polyhydroxyalkanoate synthase (PhaC), a critical enzyme involved in PHA granule production in the bacterial host and which remains covalently attached to the surface of the PHA granule. When transformed into an *E.coli* strain engineered for polyhydroxyalkanoate (PHA) bead production, the functional PhaC allows for the self-assembly of intracellular PHA beads with the immunoglobulin binding proteins expressed on their surface. Based on the results of this study, these novel beads provide us with added functionalities and significantly increased immunoglobulin binding efficiency when compared to commercial standards, which could lead to an up-scaled production of novel biopolyester beads to serve as an ideal tool for immunoglobulin purification.

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## Table of Contents

<b>ABSTRACT</b> .....	<b>1</b>
<b>ACKNOWLEDGEMENTS</b> .....	<b>2</b>
<b>LIST OF TABLES</b> .....	<b>6</b>
<b>LIST OF FIGURES</b> .....	<b>7</b>
<b>ABBREVIATIONS</b> .....	<b>9</b>
<b>CHAPTER 1: IMMUNOGLOBULIN BINDING DOMAINS</b> .....	<b>10</b>
1.1 Introduction .....	10
1.2 Protein A .....	11
1.3 Protein G .....	13
1.4 Protein L .....	15
1.5 Affinity chromatography using bacterial proteins .....	17
1.6 Immobilization of proteins on PHA .....	18
1.7 Aim of this study .....	23
<b>CHAPTER 2: MATERIALS AND METHODS</b> .....	<b>24</b>
2.1 Bacterial strains and plasmids .....	24
2.2 Liquid Media .....	25
2.3 Solid Media .....	25
2.4 Antibiotic stock solutions and final concentrations .....	25
2.5 Cloning method .....	26

2.6 PHA bead production in <i>E.coli</i> .....	28
2.7 Long term storage and revival of bacterial strains .....	28
2.8 Preparation of competent <i>E.coli</i> cells .....	28
2.9 Transformation of <i>E.coli</i> cells .....	29
2.10 DNA isolation and manipulation .....	30
2.10.1 Plasmid isolation and concentration .....	30
2.10.2 DNA hydrolysis with restriction endonucleases .....	30
2.10.3 Determination of DNA fragment size and concentration .....	31
2.10.4 Agarose gel electrophoresis (AGE) .....	31
2.10.5 DNA fragment recovery from agarose gels .....	32
2.10.6 DNA ligation .....	32
2.10.7 DNA sequencing .....	33
2.11 PHA extraction, preparation and analysis .....	33
2.11.1 Cell disruption .....	33
2.11.2 Isolation of PHA granules from crude cell lysate (Ultracentrifugation).....	34
2.11.3 Detection of PHA accumulating cells using Nile-red .....	36
2.11.4 Gas chromatography-mass spectrometry analysis (GC/MS) .....	36
2.12 General methods for protein analysis .....	36
2.12.1 Protein concentration measurement .....	36
2.12.2 Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE) .....	37

2.12.3 Preparation of protein samples for SDS-PAGE.....	39
2.12.4 Protein staining with Coomassie Blue .....	40
2.12.5 Maldi-TOF mass spectrometry (Maldi-TOF/MS) .....	41
2.13 Determination of fusion protein activity on PHA beads through IgG binding assay ..	42
2.14 Purification of IgM from hybridoma supernatant .....	42
<b>CHAPTER 3: RESULTS .....</b>	<b>44</b>
3.1 Protein G-displaying PHA beads .....	44
3.1.1 Functional assessment of IgG binding domains .....	45
3.2 Protein L-displaying PHA beads .....	49
3.2.1 Functional assessment of immunoglobulin binding domains .....	50
3.2.1.1 Binding experiment with IgG from rat serum.....	50
3.2.1.2 Purification of IgM from hybridoma supernatant.....	52
<b>CHAPTER 4: DISCUSSION .....</b>	<b>59</b>
4.1 ZZ-PhaC to ZZ-Linker-ZZ-PhaC .....	59
4.2 ZZ-Linker-ZZ-PhaC-L .....	61
4.3 Summary .....	63
<b>APPENDIX .....</b>	<b>65</b>
<b>REFERENCES.....</b>	<b>69</b>

## List of Tables

	<b>Page</b>
<b>Table 1</b> Binding characteristics of antibody-binding proteins. (from Thermo-Scientific 2008)	<b>10</b>
<b>Table 2</b> Table 2: Comparison of immunoglobulin binding specificities of the type III Fc receptor protein G and the type I receptor protein A. (from Fahnestock, 1987)	<b>14</b>
<b>Table 3</b> Bacterial strains used in this study	<b>24</b>
<b>Table 4</b> Plasmids used in this study	<b>24</b>
<b>Table 5</b> Antibiotic stock solutions and final concentrations.	<b>25</b>



## List of Figures

	<b>Page</b>
<b>Figure 1</b> Schematic diagram visualizing the PHA granule and associated proteins, including the polyester synthase or PhaC. (from Rehm, 2003)	<b>20</b>
<b>Figure 2</b> SDS-PAGE gel analysis of proteins bound to ZZ-PHA granules or protein A-Sepharose after elution (from Brockelbank et al., 2006)	<b>22</b>
<b>Figure 3</b> Vector map of the ZZ-linker-ZZ-phaC-GB13 and ZZ-linker-ZZ-phaC-L constructs	<b>27</b>
<b>Figure 4</b> Diagram of the PHA bead extraction process	<b>35</b>
<b>Figure 5</b> Fluorescence microscopy image of ZZ-linker-ZZ-PhaC-GB1 <sub>3</sub> beads produced in <i>E. coli</i>	<b>44</b>
<b>Figure 6</b> Binding experiment of 50 mg beads with 5mg IgG from human serum	<b>46</b>
<b>Figure 7</b> Binding experiment of 50 mg beads with 1mg IgG from goat serum.	<b>47</b>
<b>Figure 8</b> Unbound and elution fractions of the goat IgG binding experiment on SDS-PAGE	<b>48</b>
<b>Figure 9</b> SDS-PAGE of isolated and purified PHA beads displaying the immunoglobulin binding domain of protein L via covalent attachment to the PHA synthase	<b>50</b>
<b>Figure 10</b> Binding experiment of 50 mg beads with 1mg IgG from rat serum	<b>51</b>
<b>Figure 11</b> Binding experiment of 100 mg beads with 1 ml crude hybridoma supernatant.	<b>53</b>

<b>Figure 12</b>	SDS-PAGE analysis of unbound and elution fractions of the binding experiment with crude hybridoma supernatant	<b>54</b>
<b>Figure 13</b>	Immunostrips obtained from the half strip lateral flow assay. SDS-PAGE of isolated and purified AL-beads.	<b>56</b>
<b>Figure 14</b>	Apparent absorption (A <sub>app</sub> ) or band intensity of the bands formed on immunostrips obtained from half strip lateral flow assay	<b>57</b>
<b>Figure 15</b>	Protein concentration of elution fractions obtained from binding experiment of 50mg PHA beads with 3mg IgG from human serum	<b>60</b>
<b>Figure 16</b>	SDS-PAGE of isolated and purified AL-beads	<b>61</b>
<b>Figure 17</b>	SDS-PAGE of isolated and purified AL-beads with and without the addition of protease inhibitor	<b>62</b>

**Abbreviations**

<b>°C</b>	<b>Degree Celsius</b>
<b>3HB</b>	<b>3-hydroxybutyrate</b>
<b>AGE</b>	<b>Agarose Gel Electrophoresis</b>
<b>BSA</b>	<b>Bovine Serum Albumin</b>
<b>DMSO</b>	<b>Dimethyl sulfoxide</b>
<b>FM</b>	<b>Fluorescent microscopy</b>
<b>GC/MS</b>	<b>Gas chromatography mass spectrometry</b>
<b>Ig</b>	<b>Immunoglobulin</b>
<b>kDa</b>	<b>Kilo Daltons</b>
<b>Maldi-TOF/MS</b>	<b>Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry</b>
<b>PBS</b>	<b>Phosphate buffered saline</b>
<b>PHA</b>	<b>Polyhydroxyalkanoate</b>
<b>PhaC</b>	<b>PHA synthase</b>
<b>PHB</b>	<b>Polyhydroxybutyrate</b>
<b>SDS-PAGE</b>	<b>Sodium dodecyl sulfate gel electrophoresis</b>
<b>WT</b>	<b>Wild-type</b>