Development of immuno-reactive reagents using the IgG-binding domain of Protein G and the surface layer (S-layer) protein of *Caulobacter crescentus*

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**ABSTRACT**

The immunoglobulin G (IgG) binding protein (Protein G) is frequently the reagent of choice for immuno-reactive protein isolation and immuno-reactive assays as it is robust against denaturing or non-denaturing conditions of test systems and IgG molecules. Therefore, efforts to develop Protein G substitutes for reagents in these systems have been focused on the native protein G domain. Caulobacter crescentus produces a homologous protein (Protein A), but not like Protein G, subunits are secreted into the extracellular matrix of the bacteria. Due to the use of this system, Protein G can be expressed in Caulobacter and used as a substitute. Several attempts have been made to develop Protein G substitutes for reagents in these systems. One of these attempts used the Protein G-binding domain (GB1) to create an in vitro display system based on an S-layer protein. GB1 was cloned into a plasmid and the expression of GB1 was confirmed by western analysis to determine the amount of GB1 protein secreted into the extracellular matrix. The results show that the expression of GB1 is not only successful, but also that it is hydrophilic in character—very similar to the native Protein G. The results also show that the expression of GB1 is more cost-effective than Protein A beads and exhibit a broader species and IgG subclass range than its competitor *Caulobacter crescentus*. The binding capacity of the display constructs was comparable to Pansorbin beads, and the expression of GB1 is more cost-effective than Protein A beads. Success can open the possibility of a low cost alternative to Protein G beads and exhibit a broader species and IgG subclass range than Protein A beads.

**INTRODUCTION**

The Caulobacter S-layer has a remarkable capability for displaying large volumes of heterologous (foreign) protein while maintaining the ability to display protein G. Success can open the possibility of a low cost alternative to Protein G beads and exhibit a broader species and IgG subclass range than its competitor *Caulobacter crescentus*. The binding capacity of the display constructs was comparable to Pansorbin beads, and the expression of GB1 is more cost-effective than Protein A beads. Success can open the possibility of a low cost alternative to Protein G beads and exhibit a broader species and IgG subclass range than Protein A beads.

**RESULTS**

### Towards a solution: Can a “spacer” peptide improve IgG binding to GB1 or increase levels of recombinant S-layer secretion?

Candidate spacer: MUC1 anti-cancer vaccine antigen.

### Measuring the amount of rabbit IgG binding to the various S-layer GB1 constructs

A quantitative ELISA assay was devised to quantitate the amount of IgG bound per mg of cell and per mg dry weight of cells. The expression of Protein A in *Caulobacters* was measured and compared to Protein G beads.

### Towards a solution: Can a “spacer” peptide improve IgG binding to GB1 or increase levels of recombinant S-layer secretion?

**MUC1 “A”**

(Low pH extract of S-layer protein from equal amounts of cells)

**MUC1 “B”**

So tandem linear repeats of MUC1 “A” or “B” up to 3X (673AA’s) are remarkably well secreted and displayed in the S-layer.

**“Second Generation” display constructs created a protein scaffold (MUC1B) for GB1 in the Caulobacter S-layer and greatly improve secretion and the antibody binding function of GB1.

**RESULTS of second generation:**

The addition of a few AA on the ends of GB1 increased expression somewhat but the addition of the MUC1B segments strikingly increased expression. The multimerized GB1-G-M-G-M is an addition of 265 AA, but has near wild type expression levels and even by western analysis clearly binds more antibody.

**CONCLUSIONS/COMMENTS**

It seemed unlikely that the solution to poor secretion and functioning of a short segment could be fixed by display of tandem repeats of it, but in fact it worked very well.

The MUC1 spacer peptide was key to this success. We don’t know why it is secreted so well, but it is hydrophilic in character—very similar to the S-layer.

The display of 3 protein G binding domains interspersed with spacers is similar to what is found in the native Protein G.

In addition to binding more IgG than Pansorbin cells, Protein G display allows binding of a broader range of species and IgG isoforms.

This low cost approach to protein G display is useful for IP assays but will also be applicable for a variety of other biotech applications.