Engineered Proteins for Binding and Organization of Inorganic Particles

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Abstract:

The focus of this project is the exploitation of the self-assembly properties of proteins to arrange inorganic compounds in controlled and predictable patterns. This involves the genetic engineering of the *Escherichia coli* DNA binding protein, *lac* repressor (LacI), such that it binds to inorganic compounds without significant functional loss. LacI binds with high affinity to sequence-specific regions of double stranded DNA (dsDNA), referred to as the *lac* operator (*lacO*). The addition of an inorganic binding motif would allow for the assembly of a DNA-protein-nanoparticle structure at the *lacO* within a complex dsDNA scaffold. Inorganic binding can be incorporated into the protein by insertion of specific polypeptide binding sequences into a previously identified permissive site in the LacI protein.

The sequence for an inorganic silica binding motif was inserted into a permissive site in LacI, following the 317th residue. The LacI derivatives were then assayed for *in vivo* DNA binding function and protein expression. A β-galactosidase assay determined that the LacI-silica binding derivative maintains strong DNA binding capability, and western blot analysis showed good protein expression.

Introduction:

Lac repressor controls expression of the *E. coli* lactose metabolism genes. This protein possesses a high binding affinity for a double stranded DNA sequence identified as the *lac* operon. When bound to *lacO*, LacI inhibits the transcription of the *lac*Z, *lac*Y, and *lac*A genes, which encode for the proteins β-galactosidase, permease, and transacetylase, respectively. A derivative of LacI, with the ability to dually bind to DNA at a programmed location and to bind to silica, would allow for the highly controlled placement and assembly of an important material with interesting electrical properties. This technology has the potential to be highly useful in low range nanoscale 'bottom-up' fabrication.

A silica binding dodecapeptide (denoted QBP3) was incorporated into a LacI derivative, LacI-317, to endow the DNA binding protein with the ability to also bind to inorganic silica. LacI-317 contains a 31-codon insertion following the 317th amino acid residue, a result of Tn/*lac*Z/in mutagenesis, as previously described [2]. This LacI derivative maintains DNA binding functionality and thus the sequence location following the 317th amino acid was identified as a "permissive" site, a site in the protein that tolerates additional sequences without loss of protein function [3]. Insertion of the silica binding sequence into the LacI-317 gene was made possible by a unique *Bam*HI restriction site located in the 31-codon (93 bp) insert.

Procedures:

The Tn/*lac*Z/in-mediated insertion mutagenesis of LacI-317 was performed on a pTrc99A derivative carrying the *lacI*^{q} gene [3]. A derivative of this plasmid that contained the mutant *lac*I allele for LacI-317 was used as a cloning vector. This plasmid is referred to as *plac*I-317::*i*31. The silica binding peptide sequence, QBP3 (Leu-Pro-Asp-Trp-Trp-Pro-Pro-Pro-Gln-Leu-Tyr-His) was originally identified in the phage display library. PCR primers (5'TTCGCAATTCC TTTAGATCTACCTTTCTATTCTCACTCT3' and 5'ACT TTC AAC AGTTTCGGCCAGATCTCCACC3') were designed to amplify the QBP3 coding sequence with flanking *Bgl*II sites. This PCR product was purified and digested with *Bgl*II. The cloning vector was digested with *Bam*HI. Ligation of the insert into the *placI*-317::*i*31cloning vector was followed by a BamHI digest of the ligation reaction to

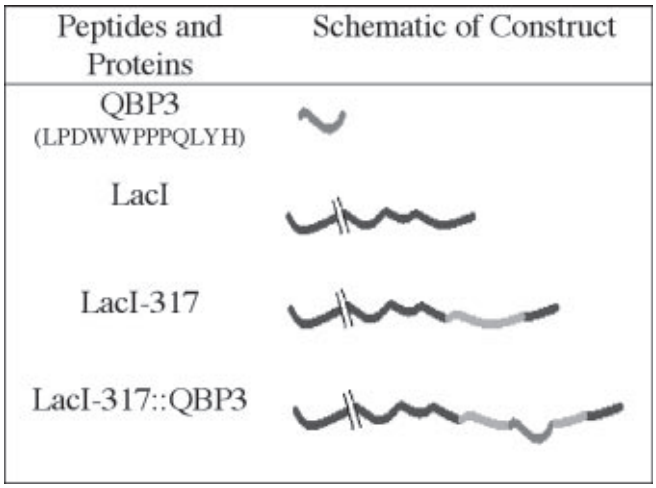

Table 1: Schematic illustration of the peptides and proteins.

eliminate religated *placI*-317::*i*31. This product was introduced to frozen competent DH5α *E. coli* (F-minus, ϕ80d*lac*ZΔM15Δ(*lac*ZYA-argF)U169 *deo*R*rec*A1 *end*A1 *hsd*R17(r_K^-,m_K^+)*pho*A*sup*E44λ-thi-*gyr*A96*rel*A1), plated onto LB-agar plates supplemented with 100 μg/ml ampicillin, and the plates incubated overnight at 37ºC.

Individual transformants where screened for the insert by PCR using an internal primer unique to the QBP3 sequence. Transformants that resulted in a PCR product were further screened by restriction digest. The binding sequence contained two unique *Bse*RV restriction sites not found in the cloning vector, *placI*-317::*i*31. Plasmids where purified using the QIAGEN kit and digested with *Bse*RV. Analysis by agarose gel electrophoresis reveals digested plasmids containing the insert as a linear fragment while plasmids without the insert run as uncut plasmids. Plasmids identified as containing the insert, *placI*-317::*i*31::QBP3, were transformed into frozen competent CSH140 *E. coli* (F128*lac*I/*ara* Δ(*gpt-lac*)5). The corresponding protein, LacI-317::QBP3 (see Table 1) was assayed for DNA binding functionality *in vivo* in a β-galactosidase activity. Since LacI represses expression of β-galactosidase, the ability of the LacI mutant to bind to the *lac* operon is inversely proportional to β-galactosidase activity levels. The assay was carried out as described by Kleina and Miller [3] with minor modifications. Protein expression was determined by western blot analysis, and was carried out as previously described [3], with minor modifications. The primary antibody used was IgG mouse monoclonal antibody against LacI.

Results and Conclusions:

Data from the insertion screening by PCR and *Bse*RV restriction digest identified two transformants as containing the insert (Figure 1). It was determined from the β-galactosidase assay that the mutants LacI-317::QBP3 maintained excellent DNA binding (see Table 2). The western blot reveals that the mutants show significant protein expression levels, with the LacI-317::QBP3 mutant protein running at a slightly higher molecular weight compared to LacI-317 and the wild type, as expected (Figure 2).

Future Work:

The continuation of this project includes confirmation of the proper insert by DNA sequence analysis, and assaying for inorganic silica binding capability. These developed techniques could be extended to insert other inorganic binding sequences, as well as inserting the sequences into other identified permissive sites in the LacI protein.

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References:

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[2] Manoil, C., Bailey, J. J. Mol. Biol. 1997, 267, 250-263.

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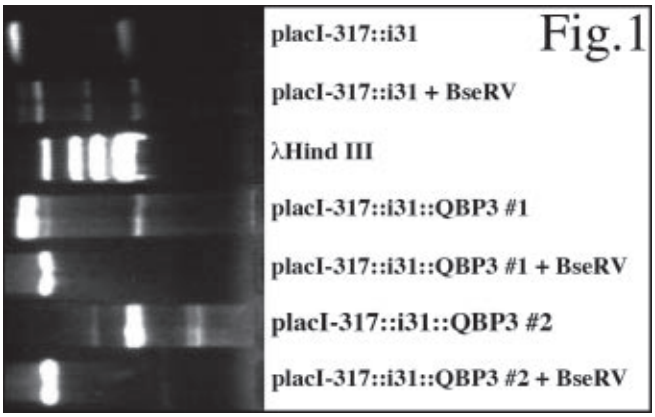

Mutant tested in CSH140	β-Gal Units
$LacI^+$ (pTrc99A)	2.45
LacI-317	-1.29
LacI-317::QBP3 #1	1.28
LacI-317::QBP3 #2	1.35
CSH140	1911.64

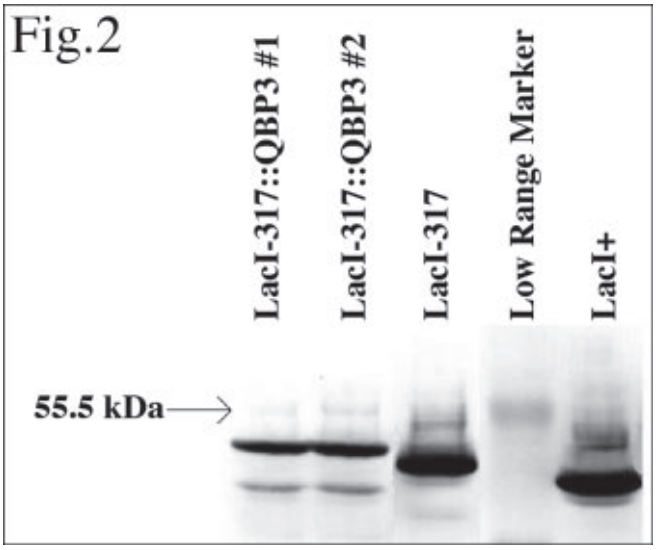

Figure 1, top: Agarose gel electrophoresis of restriction digest with BseRV.

Table 2, middle: β-galactosidase activity assay.

Figure 2, bottom: Western blot analysis.