Use of Engineered Proteins for Organization of Nanostructures

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Abstract

The *Escherichia coli lac* repressor (LacI) is a deoxyribonucleic acid (DNA)-binding protein that regulates the production of proteins involved in lactose metabolism. Permissive sites within LacI have been previously identified, where short peptide sequences can be inserted without affecting the normal function of the protein. The sequence for the inorganic silica binding motif, QBP3, was inserted into a permissive site at residue 338 of the *E. coli lac* repressor, endowing LacI with the ability to bind both DNA and inorganic silica. After PCR screening for the QBP3 insert, six candidates were sequenced. Two were chosen for continued characterization. Western blot analysis of these constructs showed good protein expression, and β-galactosidase assays indicated LacI clones maintained normal function. The constructs showed some binding to fine silica and minimal binding to coarse silica. These analyses suggest the insertion at residue 338 binds less favorably to silica than the previously isolated QBP3 insertion at residue 317. Engineered proteins like these, that bind both DNA and inorganic compounds, can be utilized to arrange nanostructures in complex predictable patterns, using DNA as a scaffold.

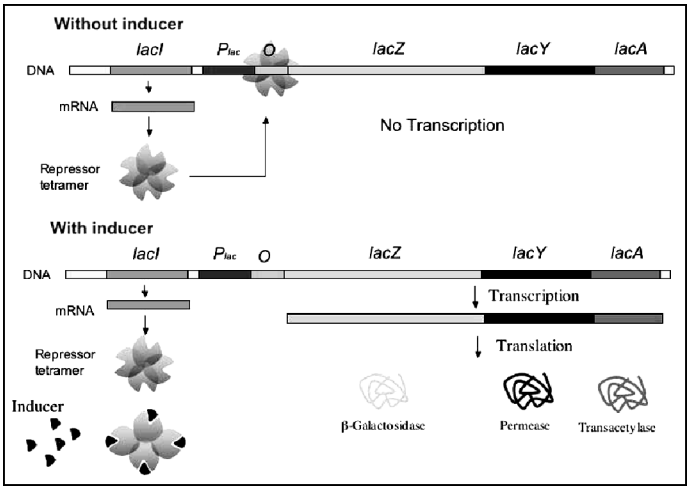

Figure 1: Diagram describing the function of the lac repressor (LacI).

Introduction

The expression of proteins involved in lactose metabolism in *Escherichia coli* are regulated by the DNA-binding protein *lac* repressor (LacI). LacI normally functions as a tetramer which binds to the *lac* operator (Figure 1). RNA polymerase binding is inhibited, repressing lac transcription and protein production. In the presence of lactose, allolactose binds to the tetramer inducing a conformational change. This new shape cannot bind to the *lac* operator, so transcription of the lactose metabolism genes occurs. Permissive sites have been previously identified within LacI [1]. These sites are permissive because short sequences can be inserted there without affecting the normal function of the protein. QBP3, a known amino acid sequence with the ability to bind silica [2], was inserted into the permissive site at residue 338 of LacI, endowing LacI with the ability to bind both DNA and silica. Expression, function and silica binding capability of the Lac-I derivatives were characterized.

Proteins with this dual binding ability can use DNA as a scaffold to organize nanostructures in complex predictable patterns. As more proteins are engineered to bind DNA and inorganic compounds, organization of complex nanostructures will become a more efficient process.

Experimental Procedure: Construction of LacI-338::QBP3

A plasmid with the lacI-338::i31 gene, *plac*I-338::i31 [1], was used as a cloning vector and digested with the restriction enzyme BamHI. The coding sequence for the silica binding motif, QBP3 (Leu-Pro-Asp-Trp-Trp-Pro-Pro-Pro-Gln-Leu-Tyr-His), was PCR amplified using engineered primers (5'TTCGCAATTCCTTTAGATCTACCTTTCTATTCTCACT CT3' and 5'ACTTTCAACAGTTTCGGCCAGATCT CCA CC3') which were designed to amplify the QBP3 coding sequence and introduce BglII restriction sites. The PCR product was purified via ethanol precipitation and digested with BglII. BglII and BamHI restriction sites leave identical overhangs which facilitated the ligation of QBP3 and *plac*-338::i31. To minimize ligation of the vector without the QBP3 insert, a background BamHI digest followed the ligation. Reactions were transformed into DH5α competent *E. coli* and plated onto LB plates, supplemented with 100 μg/ml ampicillin, and incubated overnight at 37ºC.

90 reactions were screened by PCR with the engineered primers QBPINT and 3'LacIR, which bind within and downstream of the QBP3 insert. Six candidates were further screened by sequencing. QBP3 contains a BseRI restriction site not present

in the vector. Two candidates were cut with BseRI to confirm the insertion, and chosen for further characterization (denoted 29 and 38 in the figures).

Characterization of LacI-338::QBP3

Protein expression of the clones was determined by western blotting, using the monoclonal anti-LacI primary antibody. β-galactosidase assays were carried out as described by Kleina and Miller [3] to determine DNA binding activity, in high and low plasmid copy number *E. coli* strains, CSH140 and BN29, respectively.

Silica binding assays were done with cell extracts containing various LacI derivatives supplemented with fine or coarse silica powders. Assays were incubated for 10 minutes with rotation at room temperature, then harvested. The ability of protein to bind to silica (indicated by fractionation to the pellets) was measured. Protein was detected using the LacI antibody on a Western blot.

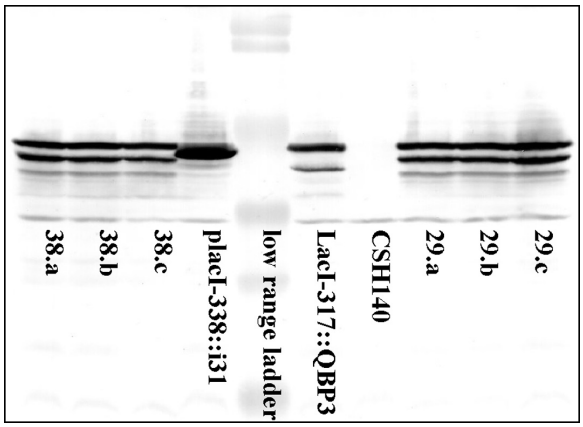

Figure 2: Western blot analysis of protein expression.

Results and Conclusions

Two LacI-338::QBP3 clones were isolated and characterized. The LacI-338::QBP3 derivatives displayed good protein expression [Figure 2]. Smaller LacI-related bands were present in the Western blot, suggesting these proteins were somewhat more susceptible to degradation than the positive controls. β-galactosidase assays showed good repression activity was maintained in both strains, indicating DNA binding by LacI-338::QBP3 (Table 1). The clones displayed some fine silica binding and even less affinity for coarse silica (Figure 3). The silica binding assays also suggested the insertion at residue 338 binds less favorably to silica than the insertion at residue 317.

Future Work

It will be of interest to determine whether the affinity for binding various inorganic compounds varies by motif insertion at different locations, as seen here. Also, this project can be continued with the characterization of QBP3 and other inorganic binding sequence inserts into various DNA-binding proteins. As more constructs are built and characterized, organization of nanostructures using these engineered proteins will become a more efficient process.

Acknowledgments

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References

[1] Nelson, B.; C. Manoil; B. Traxler; J. Bacteriol., 179, 3721-28 (1997).

[2] Oren, E.E.; C. Tamerler; D. Sahin; M. Hnilova; U.O.S. Seker; M. Sarikaya; R. Samudrala; Bioinformatics, (2007), in press.

[3] Kleina, L. G.; Miller, J. H.; J. Mol. Biol., 212, 295-318 (1990).

mutant	β-gal activity
(assayed in CSH140)	
pTrc99A (wt LacI)	0.0010
LacI-317::QBP3	0.0003
placI-338::i31	0.0005
29.a	0.0007
29.b	0.0008
29.c	0.0009
38.a	0.0006
38.b	0.0008
38.c	0.0006
(assayed in BN29)	
BN29	152.863
pTrc99A (wt LacI)	0.598
placI-338::i31	7.200
29.a	17.300
38.c	10.39

Table 1: β-galactosidase activity in Miller units.

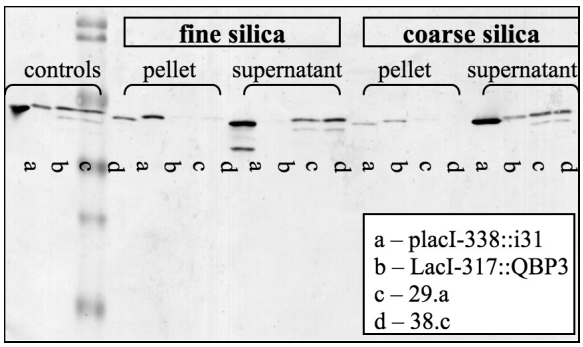

Figure 3: Western blot analysis of silica binding assays.