Enzyme

Modification of the enzyme to generate novel carbohydrate binding proteins for glycoprotein analysis

Amy Harrington[1], Roisin Thompson[1], Brendan O'Connor[1], Paul Clarke [1]

1. Irish Separation Science Cluster, Dublin City University, Dublin

Objectives:

- **The cloning, expression, purification and characterisation of a recombinant prokaryotic glycolytic enzyme**
- **The site specific mutagenesis of the prokaryotic glycolytic enzyme to generate a novel recombinant carbohydrate binding protein**
- **The characterisation of the binding profile of the novel recombinant carbohydrate binding protein**
- **Random Mutagenesis of the glycolytic enzyme to study its mechanism of action and the possible generation of more novel carbohydrate binding proteins**
- **Immobilisation of the protein to various surfaces including monoliths, polyHIPES and gold nanoparticles to produce advanced bioanalytical platforms**

1. Introduction:

The overall objective of this project is to generate novel carbohydrate binding proteins for use in glycoprotein analysis which are amenable to large scale production. The approach used here is the modification of prokaryotic glycolytic enzymes. Their enzymatic activity will be eliminated while hoping they still retain their binding capabilities. These proteins will be immobilized onto different surfaces to generate advanced bioanalytical platforms which will have huge commercial potential in the field of glycoanalysis.

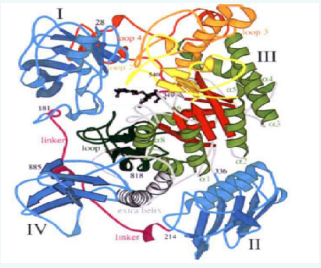


Figure 1: A *homologue of the prokaryotic glycolytic enzyme.* A homologue of the prokaryotic glycolytic enzyme has already been cloned and purified. Its catalytic activity has been removed and it has been shown to bind glycans in solution [Tews *et al.* 1996, Prag *et al.* 2000]. This work has been used as a template for our site specific mutagenesis.

2. Cloning, Expression, Purification and Characterization of the prokaryotic glycolytic enzyme (G)

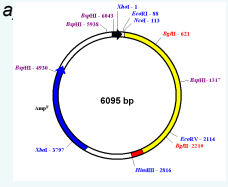
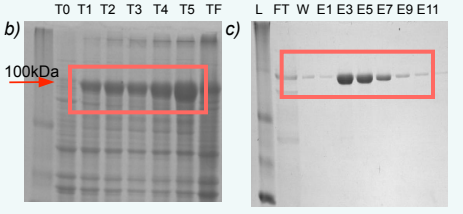
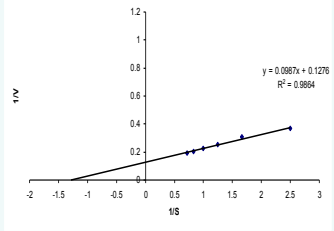


Figure 2: *a) Construct map. PCR product of the gene of interest (yellow) was inserted into a pQE60 vector. This vector adds a C terminal His tag (red) to its product. The vector also contains ampicillin resistance (blue) for selection. This product was subsequently transformed into Escherichia coli KRX for expression.*



b) Supernatant samples from E. coli cultures containing the expression vectors were taken every hour for 5 hours. SDS-PAGE was used to examine expression of the enzyme (G). c) IMAC purification of the enzyme (G).



e)

V_{max} (mM)	7.83
K_m (mM)	0.75
K_{cat} (s^{-1})	0.783
K_{cat}/K_m (1/mM.s)	1.044

d) Colorimetric Assay to test activity of the glycolytic enzyme (G). A 4-Nitrophenol linked substrate was cleaved by the enzyme to release a coloured compound which can be detected. A Lineweaver-Burke plot was generated with the results. e) Lineweaver Burke values calculated to describe activity of the enzyme (G).

3. Site Specific Mutagenesis of the glycolytic enzyme (G) to remove activity and generate a novel carbohydrate binding protein (ΔG)

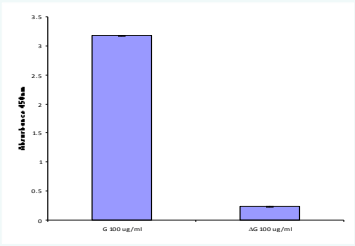


Figure 3: *Site Specific Mutagenesis of recombinant enzyme G results in a significant reduction of catalytic activity as determined by the colorimetric assay.*

4. ELLA analysis detects binding of carbohydrate binding protein (ΔG) to sugar residues

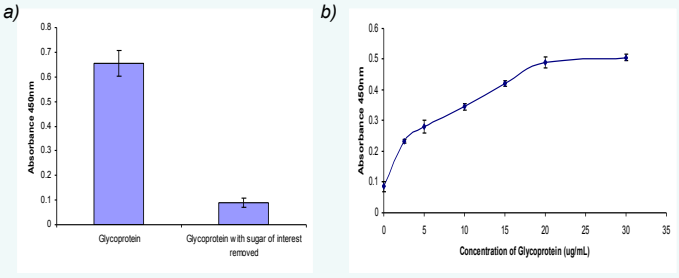


Figure 4: *a) ELLA used to detect binding of ΔG to sugar of interest. A sandwich assay format was used as direct detection was not successful. b) Binding of* ΔG to sugar of interest is concentration dependent

5. Future Work

- Random mutagenesis to:
  - Study mechanism of action of the enzyme
  - Generate more novel carbohydrate binding proteins
- Immobilisation of carbohydrate binding protein (ΔG) to various surfaces including monoliths, polyHIPES and gold nanoparticles to generate advanced bioanalytical platforms.

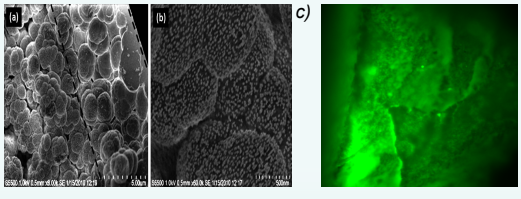


Figure 5: *a) and b) Lectin modified gold nano-particles on a polymer monolithic phase. c) GFP immobilised onto polyHIPES.*

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