The use of chitin binding proteins for glycoprotein analysis

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Introduction: The focus of the pharmaceutical industry has dramatically shifted in the past number of years. Traditional drugs were synthesised using chemical reactions have been replaced by recombinant glycoprotein molecules. These potential recombinant glycoprotein therapeutics display oligosaccharide structures on their surfaces that are recognised by their target host. The specific glycan moieties on the surface of the molecules vary dramatically and have a large impact on the efficacy of the drug. The development of bioanalytical tools to identify and separate the species of glyco-forms present in a preparation of the glycoprotein therapeutic will significantly help to advance the quality and effectiveness of recombinant glycoprotein molecules. Traditionally lectins, isolated from plants, had been used to profile sugar species displayed on glycoproteins. I have explored the use of prokaryotic chitin binding proteins (CBPs) to investigate structures on glycoproteins.

Objectives: To develop novel carbohydrate binding protein bio-ligands, based on natural chitin binding properties for the ultimate analysis of glycoprotein therapeutics.

Cloning: The chitin binding protein CBP21 from *Serratia marcescens* (Suzuki *et al*. 1998) was amplified from *S. marcescens* koln genomic DNA using PCR. The gene was subsequently cloned into an *e.coli* expression vector pQE60. This vector incorporates a poly histidine tag onto the C terminus of the protein, there is also an IPTG inducible T5/lac promoter. The vector was named pRL11.

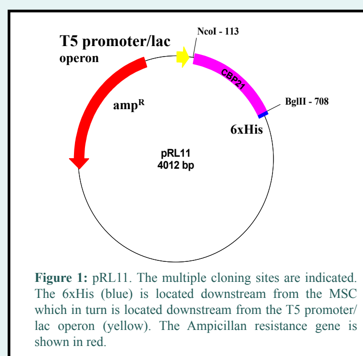

Figure 1: pRL11. The multiple cloning sites are indicated. The 6xHis (blue) is located downstream from the MSC which in turn is located downstream from the T5 promoter/ lac operon (yellow). The Ampicillan resistance gene is shown in red.

Expression: Freshly transformed cells can vary widely as to the amount of protein produced. Clonal selection was used to select a clone producing high amounts of soluble protein (Dahlroth, *et al*. 2006). KRX clone was ultimately selected for recombinant protein production. It was possible to produce 6mg of protein from a 400ml culture using this clone.

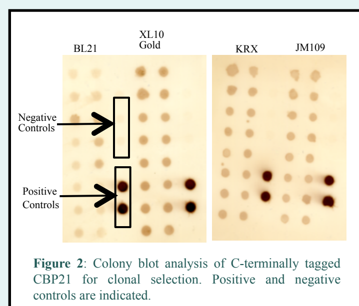

Figure 2: Colony blot analysis of C-terminally tagged CBP21 for clonal selection. Positive and negative controls are indicated.

Purification: The incorporation of a 6-His poly Histidine tag at the C-terminus of the protein allowed for purification using Immobilised metal affinity chromatography (IMAC).

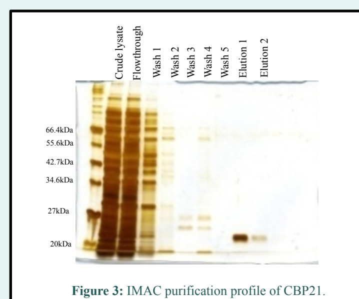

Figure 3: IMAC purification profile of CBP21.

Characterisation: Following the purification of CBP21 its activity was tested by checking the affinity of CBP21 to chitin, avicel (cellulose) and chitosan (de-acetylated chitin). Binding of the insoluble substrates was assessed in column by an incubation stage followed by washing steps. Samples were visualised using SDS-PAGE.

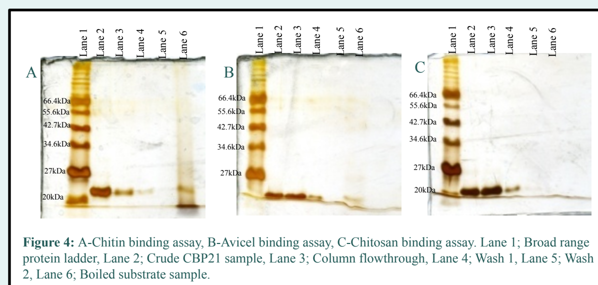

Figure 4: A-Chitin binding assay, B-Avicel binding assay, C-Chitosan binding assay. Lane 1; Broad range protein ladder, Lane 2; Crude CBP21 sample, Lane 3; Column flowthrough, Lane 4; Wash 1, Lane 5; Wash 2, Lane 6; Boiled substrate sample.

Preliminary ELLA analysis: Enzyme linked lectin assays (ELLA) were used to profile the interactions of the recombinant protein CBP21 with commercial glycoproteins.

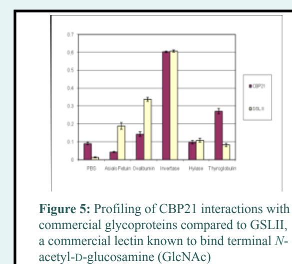

Figure 5: Profiling of CBP21 interactions with commercial glycoproteins compared to GSLII, a commercial lectin known to bind terminal *N*-acetyl-D-glucosamine (GlcNAc)

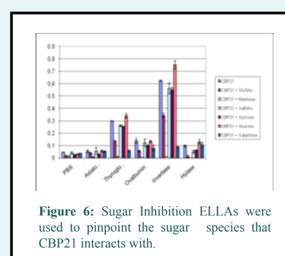

Figure 6: Sugar Inhibition ELLAs were used to pinpoint the sugar species that CBP21 interacts with.

Discussion: Further ELLA analysis must be carried out to identify the specific sugars that CBP21 interacts directly with. Interestingly it can be seen from the sugar inhibition ELLA studies that Mannose and galactose inhibit CBP21 interaction with the commercial glycoproteins to a greater extent than GlcNAc.

Future Work: ELLA analysis of CBP21 using enzyme treated glycoproteins to pinpoint the specific interactions of CBP21. Site directed mutagenesis of CBP21 to alter the binding specificity. Cloning, expression and profiling of CBP21 homologues. Further characterisation of glycoprotein binding molecules using iCT 2000, Biacore and NMR.

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