Protein from *Bacillus thuringiensis* as a Tool for Glycan Analysis and Detection

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Introduction

Changes in the structures of glycans on the surfaces of eukaryotic cells can be important biomarkers for developmental or disease states. Improved methods are needed for the detection and analysis of alterations in glycan structures. Carbohydrate binding proteins such as lectins have potential for the recognition of changes in glycan structure.

Host-pathogen interactions frequently involve the recognition of host carbohydrates by proteins of bacteria or viruses. Many bacterial toxins have evolved to interact with host cell receptors or with a specific tissue due to lectin like properties. The toxins from *Bacillus thuringiensis* have been shown to have carbohydrate binding abilities, in particular N-Acetylgalactosamine (GalNAc) has been shown to inhibit the binding of the toxin Cry1Ac. GalNAc has been shown to be an important marker in many diseases such as breast cancer and colon carcinogenesis. Moreover, changes in GalNAc glycosylation have been identified in many disorders such as cystic fibrosis, neuromuscular disorders and nephropathy. Here we describe the purification of a GalNAc binding protein of bacterial origin that may have potential in the development of diagnostic assays.

1. Results

Two truncated forms of Cry1Ac have been cloned, expressed in *Escherichia coli* and purified by Immobilised Metal Affinity Chromatography (IMAC) (Figs 1-3). The binding abilities of both proteins have been analysed in an Enzyme Linked Lectin Assay (ELLA). Fig 4 shows a comparison of the performances of the rCry1Ac proteins in comparison to commercial lectins as measured by ELLA. tCry1Ac clearly shows stronger binding to GalNAc than the commercial lectins. Inhibition assays showed that the interactions are GalNAc specific.

2. Cloning of truncated *cry1Ac* gene

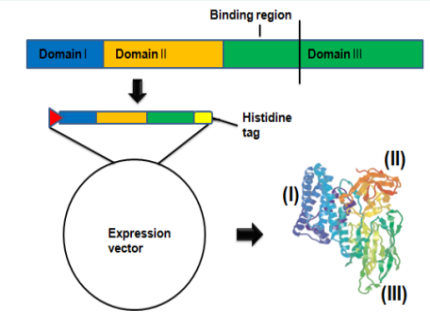

Figure.1. *Cloning of a truncated cry1Ac (tcry1Ac) gene into an expression vector. The binding region is marked on the gene and is on the domain III of the protein. This vector was used for both the tCry1aAc and the binding domain (CryD3).*

3. The Expression of tCry1Ac in *E. coli*

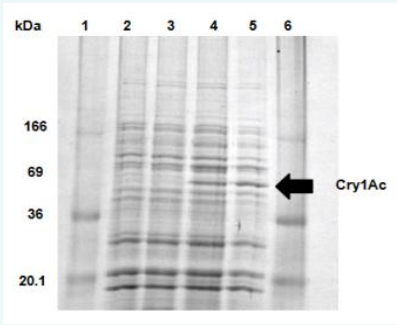

Figure.2. *Expression of tCry1Ac in E. coli.*
1, 6*; Broad range protein ladder,* ***2****; 2 hours post induction,* ***3****; 3 hours post induction,* ***4****; 4 hours post induction,* ***5****; overnight post induction*

4. Purification of tCry1Ac by IMAC

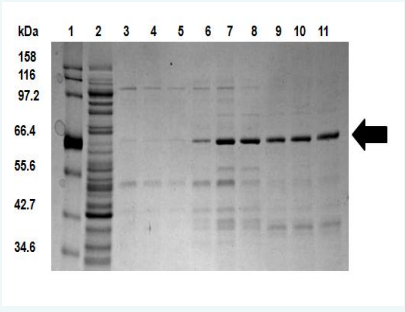

Figure.3. *Purification of tCry1Ac by IMAC*
1*; Protein marker (broad range),* ***2****; Flow through,* ***3****; 20mM imidazole wash,* ***4,5,6****; 40mM imidazole wash,* ***7,8,9,10****; Elution 300mM imidazole*

5. ELLA of tCry1Ac and commercial lectins

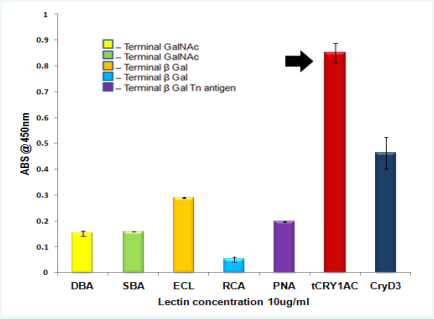

Figure.4. *ELLA showing binding of tCry1Ac, CryD3 and commercial lectins to GalNAc - BSA. The specificity of each commercial lectin is highlighted. All lectins and tCry1Ac and CryD3 are at a concentration of 10µg/mL. tCry1Ac clearly shows very strong binding to GalNAc .*

6. Affinity ELLA of tCry1Ac to GalNAc

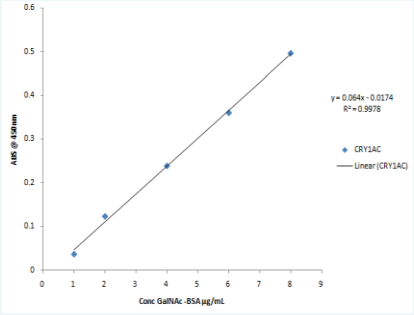

Figure.5. *ELLA showing the linear response of recombinant tCry1Ac to the GalNAc-BSA. Inhibition assays showed that the interaction is GalNAc specific.*

Conclusions and future work

We have shown that truncated forms of the *Bacillus thuringiensis* toxin (Cry1Ac) have been cloned, expressed, purified and their binding activity assessed on ELLA's. Significantly the recombinant tCry1Ac has a higher specificity for GalNAc than commercial lectins while the binding domain CryD3 retains the specificity for GalNAc also. This is very significant as the binding domain of Cry1Ac is 16kDa, which may be easier to manipulate than the larger tCry1Ac (69kDa). ELLA's will be carried out on the recombinant tCry1Ac and CryD3 to examine the stability of the proteins. Further studies will be carried out on the affinity and specificity of tCry1Ac and CryD3 by mutagenesis. It is planned to use these carbohydrate binding proteins in the analysis and detection of O-glycosylation in host-pathogen interactions and pathogenesis. These proteins could also be immobilised onto novel bio-affinity surfaces which would prove extremely useful in the detection, analysis and separation of glycans and glycoproteins.

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