Affinity Tagging & Purification Of The Fucose Binding LecB Protein.

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Introduction

The fucose binding LecB protein is one of two identified lectins produced by the opportunistic pathogen *Pseudomonas aeruginosa* (PA01) and is implicated in contributing to its virulence. A large number of homologous proteins have been identified in other bacterial species that exhibit extremely high sequence identity and similarity to LecB. However, key amino acid residues known to participate in fucose binding in LecB are altered in many of these proteins. Some of these proteins have been shown to exhibit altered sugar specificities while others are as yet uncharacterised. The existence of such homologues suggests the sugar binding specificity of the LecB protein could potentially be further diversified through mutagenesis to generate novel biomolecular recognition molecules for glycoprotein characterisation and purification applications.

Project Objectives

- To clone the wild type *lec*B gene into suitable expression vectors to facilitate expression of the protein in *Escherichia coli* with affinity tags.
- To optimise the expression of the wild type LecB protein in *E. coli* and its subsequent purification utilizing the incorporated affinity tags.
- To functionally and structurally characterise the wild type affinity tagged LecB protein to assess the impact of the incorporated affinity tags.
- To alter the sugar binding specificity of the LecB protein through directed and random mutagenesis strategies.

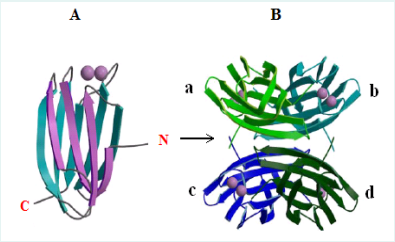

Figure 1: (A) Crystal structure of LecB monomer. The sugar binding site of each monomer binds two calcium ions, indicated as purple spheres, which then participate in fucose binding. Protein N and C-termini are indicated.
(B) Native protein composed of four identical subunits a, b, c and d. The structure is maintained by protein-protein interactions. The (a) subunit is involved in interactions with subunits (b) and (d) (Mitchell E *et al.* Proteins; 2005).

Cloning & Affinity Tagging Of Wild Type LecB

The wild type *lec*B gene was amplified from PAO1 genomic DNA and cloned into an *E. coli* expression vector. This vector incorporates a 6HIS affinity tag at the C-terminus of the gene and places it under the transcriptional control of a strong IPTG inducible *ptac* promoter. The resulting plasmid was called pLecB3 (see Figure 2).

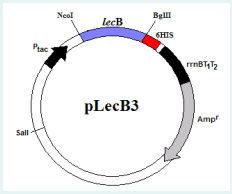

Figure 2: Plasmid map of pLecB3

Expression & Purification Of Affinity Tagged Wild Type LecB

The pLecB3 expression vector was introduced into *E. coli* stain BL21 (DE3). Protein expression and subsequent affinity purification utilizing Ni-NTA agarose (IMAC) resin were optimised. High yields of LecB protein (400µg/100ml culture) were obtained with a high level of purity (see Figure 3).

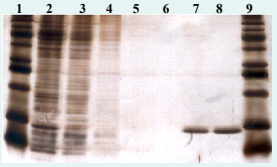

Figure 3: SDS-PAGE Analysis Of Purified LecB. Silver stained 20% Gel. Lane [1 & 9] MW Standards [2] Crude Cell Extract, [4, 5 & 6] 80mM, 80mM & 120mM Immidazole washes respectively, [7 & 8] elution fractions 1 & 2 respectively showing highly purified LecB protein at expected size of 12.9kDa.

Funtional & Structural Characterisation Of Affinity Tagged Wild Type LecB

Functional Characterisation

The functionality of the C-terminally 6HIS tagged LecB protein was assessed by hemagglutination assay and failed to display activity. As this assay is not only dependent on the capacity to bind sugar but on also on the multivalency of the protein it was possible that the C-terminally added 6HIS affinity tag may have only affected the ability of the protein to form a tetrameric (or minimally a dimeric) structure and not the sugar binding activity.

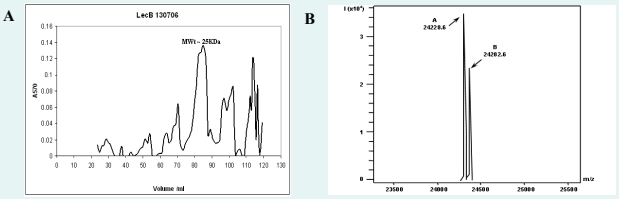

Figure 4: (A) FPLC elution profile of C-terminally affinity tagged wild type LecB. The predominant peak is indicative of a MW of 25kDa, the expected size of a LecB dimer. **(B)** ESI-mass spectrum of the infused 10.00µM LecB in Acetonitril/Water/NH3 (25/75/0.2/0 acquired on ESQUIRE-LC MS. The major peak in the spectrum (A+28) deconvolves to a mass of 24228.6 Da which corresponds to a dimer.

Structural Characterisation

The effect of the C-terminal 6HIS tag on the structure of the LecB protein was assessed by gel filtration. As can be seen from Figure 4A this confirmed that the C-terminally tagged LecB protein primarily formed a dimer and this dimer was also observed in mass spectroscopic analysis (see Figure 4B).

Considering the C-terminal positioning of the 6HIS tag we propose that the dimer formed has an (a-d) subunit conformation rather than an (a-b) subunit conformation (see Figure 1B). Such a dimer might be expected to be inactive in binding fucose as the C-terminus of the (b) subunit plays a role in fucose binding by the (a) subunit and vise versa. Overall this suggests the minimal functional unit for LecB would be an (a-b) dimer.

Alternative Tagging Of The LecB Protein

Work was undertaken to introduce a 6HIS tag at the N-terminus of the LecB protein. This work has just been completed. Following purification this N-terminal tag can also be proteolytically cleaved off to obtained unmodified LecB. Initial expression runs and crude purifications have been successfully completed (see Figure 5) but functionality has yet to be tested. If successful mutagenesis of the LecB will then be undertaken to diversify its sugar binding specificity.

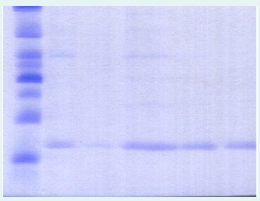

Figure 5: Initial purification of N-terminally 6HIS tagged LecB.

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