Localisation of the LPS-binding motif in GBP and its Human Homologue and Recombinant Expression

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ABSTRACT

Galactose binding protein (GBP) is a pattern recognition receptor in the innate immune system of horseshoe crabs. It contains six beta-propeller domains of which domain 1 (GBP1) harbours a lipopolysaccharide (LPS)-binding motif. The last domain hTectonin (hTec6), a possible human homologue of GBP, also contains an LPS-binding motif. GBP1 and hTec6 were subcloned and expressed in \(E.\ coli\). Rather than the expected size of 4.3 kDa, recombinant GBP1 was purified as an 18 kDa or 75 kDa protein, depending on purification conditions. Mass spectrometric analysis of the 75 kDa band revealed a bacterial enzyme responsible for resistance to polymyxin and cationic anti-microbial peptides. The presence of this enzyme suggests that the host bacteria are responding to the proposed antimicrobial properties of GBP1. This hypothesis needs further validation by expressing GBP1 in a eukaryotic expression system or \textit{in vitro} transcription and translation (TnT).

INTRODUCTION

Galactose binding protein (GBP) is an important pattern recognition receptor (PRR) in the innate immune system of horseshoe crabs (\textit{Carcinoscorpius rotundicauda}). \textit{In vivo}, it exists as a 26kDa monomer, a 52kDa dimer and 18kDa 2/3mer. GBP recruits other immune proteins to the site of infection of the pathogen by forming an interactome with other PRRs such as C-reactive protein, ficolins (Ng \textit{et al.}, 2007), lipopolysaccharide binding protein (Chiou \textit{et al.}, 2000), hemocyanin (Jiang, NUS PhD thesis, 2007), as well as Gram-negative and Gram-positive bacteria (Chen \textit{et al.}, 2001). Despite showing many interactions in the hemolymph of horseshoe crabs, a human homologue of GBP has yet to be found. Computational analysis has identified a possible homologue, hTectonin (accession number BC053591) which contains a lipopolysaccharide (LPS) binding motif (Frecker \textit{et al.}, 2000) in its last tectonin domain (Low \textit{et al.}, unpublished data). LPS is found on the surface of Gram- negative bacteria and consists of a bioactive centre, lipid A, to which sugars are attached (Takada and Kotani, 1989). GBP also contains an LPS binding motif in domain 1 of its 6 tectonin domains. This project focuses on cloning and recombinant expression of the last tectonin domain of hTectonin (hTec6) and domain 1 of GBP (GBP1).

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MATERIALS AND METHODS

GBP1 and hTec6 were subcloned from GBP and hTec cDNA respectively into a bacterial plasmid, pET22b. Expression was induced with 0.1, 0.2, 0.3 and 0.4 mM of IPTG over 2, 4 and 6 hours. Talon-Sepharose beads conjugated with Cobalt (Clontech) and nickel-Sepharose (GE Healthcare) were used to purify the recombinant proteins from the soluble fraction of bacterial cell lysate. Tricine SDS-PAGE gel was run according to Nature protocols (Schägger, 2006). Anti-GBP and anti-His antibody were used as immunoprobes to detect GBP1 and hTec6 for Western blots, respectively.

RESULTS AND DISCUSSION

GBP1 and hTec6 were successful expressed in E. coli (Fig. 2).

Figure 1. Predicted domain architecture of GBP1 (a) and hTec6 (b). Light blue boxes highlight the tectonin domains that were cloned. GBP1 contains 36 amino acids and hTec6 contains 37 amino acids. Figures from http://smart.embl-heidelberg.de.

Figure 2. Expression of GBP1 (a) and hTec6 (b) induced at different concentrations of IPTG over different time points.
While the size of hTec6 appeared as expected (4.3kDa), GBP1 seems to have oligomerised into a tetramer of 18kDa despite the fact that the electrophoretic resolution was performed under reducing conditions. GBP1 was then purified using nickel-Sepharose affinity chromatography, resulting in impure elutions despite low amounts of imidazole in the binding and wash buffers (Fig 3a). The NaCl concentration was doubled to 1 M to reduce non-specific ionic binding to the beads and this surprisingly produced a 75 kDa band in the eluted fractions (Fig. 3c) which was subsequently sent for mass spectrometric analysis.

The mass spectrometric analysis (Fig. 4) did not show any GBP1 fragments, but a bacterial enzyme that functions in a pathway whose end-product is a sugar which is attached to lipid A for resistance to polymyxin and cationic anti-microbial peptides. Since the LPS binding motif in GBP1 is basic/cationic (Frecer et al., 2000), the bacterial host may be producing this enzyme in response to the antimicrobial properties of GBP1.

**CONCLUSION AND FUTURE PERSPECTIVES**

The possible oligomerisation and covalent interaction with bacterial proteins with the GBP1 is an intriguing observation. To elucidate the mechanisms of these interactions, future studies would use eukaryotic expression systems or in vitro transcription and translation (TnT). hTec6 has also been subcloned into prokaryotic expression system with the aim of characterising its lipid A or LPS binding function. Parallel investigations of GBP1 and hTec6 would provide an insight into these novel PRR domains with antimicrobial activities.
Figure 4. Peptide mass fingerprint from the p75 band (Fig. 3b). The peptide mass fingerprint matched the bacterial enzyme, bifunctional UDP-glucuronic acid decarboxylase/UDP-4-amino-4-deoxy-L-arabinose formyltransferase.

REFERENCES


