Improving the Recombinant Protein Expression of Human Galectin-3 in BL21 Bacterial Host System

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors NRV and FPD taken responsibility in the conception and design of the study and contributed substantially in compiling literature sources and drafting the manuscript. Authors HB and SM involved in development of methods and data analysis. Authors DD and FPD have provided critical revision of the article for important intellectual content. Authors TR and SSM has checked the references. Author PKV have given final approval of the version to be published. All authors read and approved the final manuscript.

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ABSTRACT

Background: Regardless of the broad explore in the territory of glycobiology concerning structure and capacity of glycans, lectins and glycosylation forms, numerous viewpoints are still left unexplored.

Aim: In this study, we analyzed the effect of shuttle vector on the secretion of human galectin recombinant protein.

Methods: The galectin was expressed in E. coli BL21 by growing the bacterial culture in SOC medium and purified by nickel-based affinity chromatography due to its His-tag.

Results: After cell lysis the protein was identified as a single 29 KDa band by 12% SDS-PAGE.

Conclusion: Characterization studies clearly revealed that the purified protein was indeed galectin 3.
1. INTRODUCTION

Galectin-3 is widely expressed in adult tissues, particularly on and secreted by activated macrophages, monocytes and adipocytes [1]. Galectin-3 is the only member of the galectin family that can form dimers through intermolecular interactions involving the N-terminal domain. Galectin-3 dimers can cross-link cell surface glycoproteins of various cells, causing cell activation [2]. They can also mediate cell-cell and cell-extracellular matrix adhesion by serving as a bridge to bind cells together or cells to extracellular matrix proteins [3]. Extracellular functions include the modulation of cell-substrate or cell-cell adhesion, cell migration, cell activation, proliferation and survival [4]. In this way, galectin-3 is an important factor during the immune response, cancer progression, chronic inflammation and infection [5-8]. Although it is of great interest to find reliable, potent and easily available purification methods for galectin-3 from tissue sources, the quest has been mostly unsuccessful [9]. Nevertheless, finding quick and efficient method is of great interest because galectins have no signal sequence and post-translational modifications of secreted proteins, such as glycosylation and disulfide bonding [10]. Therefore, it is relatively convenient to produce recombinant galectins in E. coli for various biological studies, including inflammatory studies in physiological and pathophysiological situations [11].

2. MATERIALS AND METHODS

2.1 Plasmid Preparation

As bacterial hosts, the *Escherichia coli* strains Top10, JM109 or BL21 (Fermentas) were used. Cell pellet was harvested by centrifugation at 6,000 rpm for 15 min at room temperature. The supernatant was removed and plasmids were collected via Midi-Prep (GeneJET Plasmid, fermentas), as per the manufacturer’s instructions. Plasmids were eluted in 1 mL 1 mM Tris/HCl pH 8 or sterile ddH2O and plasmid concentration was determined by comparing the DNA concentration of 1 μL linearized plasmid with 5 μL MassRuler DNA Ladder Mix (Fermentas; Fig. 1) and measured (NanoDrop 2000, Thermo Scientific) [12].

2.2 Cloning Process

pPUZZLE vector was used, allowing easy exchange of vector components by restriction digestion and inserting different sequences. This shuttle vector was used as a vector throughout this work. The artificial sequences were exchanged by using restriction sites added via PCR to gene of interest and analysed by agarose gel electrophoresis (Fig. 2).

2.3 Transformation into *E. coli*

The first few transformations were carried out with JM109 and Top 10 strain, whereas afterwards we switched to BL21 because of its easy growth and maintenance. For each transformation (ligated empty vector as negative control), 100 μL aliquot was put on ice 15 min prior to transformation and the eluted, ligated plasmid was added to the cells. Transformation was performed using a BioRad Micropuler, a charging voltage of 2 kV and a pulse length of 4 ms. Thereafter, cells were transferred into 1 mL sterile SOC-medium for a 30 min regeneration period. Then they were plated out in aliquots (50 μL, 200 μL and remains) on selective LB-agar plates containing 25 μg mL⁻¹ Zeocin and inoculated for 24 h at 37°C [13].

2.4 Vector Transformation

Transformation of BL21 star (Invitrogen) bacteria was performed as follows: 0.5μg of pPUZZLE vector, with a His-tagged galectin-3 insert was added to 100 μl of bacteria, mixed gently and placed on ice for 30 minutes. Transformation was performed using a BioRad Micropuler, a charging voltage of 2 kV and a pulse length of 4 ms. Thereafter, cells were transferred into 1 mL sterile SOC-medium for a 30 min regeneration period. Then they were plated out in aliquots (50 μL, 200 μL and remains) on selective LB-agar plates containing 25 μg mL⁻¹ Zeocin and inoculated for 24 h at 37°C [14].

2.5 Galectin-3 Purification

Sonication, followed by triton X 100 treatment for 1 hour, cleared lysate was achieved using centrifuge and Galectin-3 was purified using Ni-NTA His-Bind Resin. 1 ml of Ni-NTA His-Bind Resin (Qiagen), per purification, was washed with 4 ml 1X Ni-NTA Bind Buffer (300 mM NaCl, 50 mM sodium phosphate buffer, 10
mM imidazole, pH 8.0), the beads were allowed to settle by gravity or centrifuged at 1000rpm for 5 minutes, and the top bind buffer layer was removed. 4 ml of cleared lysate was loaded onto the prepared Ni-NTA His-Bind slurry and mixed gently by shaking at 4°C for at least 60 minutes. This was then loaded onto a disposable polypropylene column (1-5 ml bed volume) (Pierce Biotechnology), flow through collected (aliquot taken for SDS-PAGE analysis) and 3 washes were carried out using Ni-NTA Wash Buffer (300 mM NaCl, 50 mM sodium phosphate buffer, 20 mM imidazole, pH 8.0), (aliquots of washes taken for SDS-PAGE analysis). 4x 500 μl elutions were performed with Ni-NTA Elution Buffer (300 mM NaCl, 250 mM imidazole, 50mM sodium phosphate buffer, pH 8.0) and elutions were collected in 4x 1.5 ml eppendorf tubes. Fractions were analysed using SDS-PAGE and elutions dialysed against 1L cold 1X PBS overnight (dialysis tubing size – 14.3mm, molecular weight cut off 12-14KDa). Protein determination using Pierce BCA protein assay reagent was performed and SDS-PAGE is used to verify the presence of galectin-3 [15].

2.6 SDS-PAGE

For protein gel analysis the NuPAGE (Invitrogen) Bis-Tris system was used. 15 μL sample, 4 x SDS loading dye and 2 μL Reducing Agent were heated at 99°C and 15 μL were loaded per slot. Dependent on the subsequent visualization of proteins, several different standards were used. For stain, we used 5 μL PageRuler Prestained Protein Standard for colorimetric detection (Fig. 3) [16].

3. RESULTS AND DISCUSSION

3.1 Plasmid Recovery

Plasmid DNA is qualified by isolating high copy plasmid DNA from 0.5-5 mL of overnight E. coli culture grown in LB or rich growth medium (OD_{600} = 3-5). The quality of isolated DNA is evaluated spectrophotometrically and by agarose gel electrophoresis. The purified DNA has an A_{260}/A_{280} ratio of 1.8±0.2.

3.2 Analysis of Recombinant Proteins

To obtain the protein, the engineered strains, BL21 were cultured and induced by IPTG at the concentration of 1 mM for overnight. SDS-PAGE analysis demonstrated that recombinant galectin protein was efficiently and inducible expressed in inclusion body form and could dissolve in 6 M urea. The molecular weight of the galectin protein was shown to be approximately 29 KDa as expected. According to the results of SDS-PAGE and gel image analysis, the purified protein accounted major proportion of total protein.

Fig. 1. Plasmid recovery. High-copy number plasmid DNA was isolated from overnight bacterial culture (1.5 mL, Top10, JM109 and BL21) and Purified plasmid DNA was analyzed by agarose (1%) electrophoresis.
Fig. 2. pPUZZLE vector and the human galectin-3 insert were carried out and run on 1% agarose gels. The relevant bands (indicated with arrows) were gel extracted and ligations were performed to make recombinant vector.

Fig. 3. Analysis of recombinant proteins using SDS-PAGE. His-tagged recombinant galectin-3 was extracted from BL21 cells through binding to Ni-NTA His bind resin. Aliquots of flow through (FT), washes (W1-3) and elutions (E1-5) of human galectin-3 were analysed using SDS-PAGE.

4. CONCLUSION

For over a century, the areas of nucleic acids, proteins and lipids have captured the attention of investigators worldwide. Carbohydrates, probably because they are very complex and not encoded in the genome, have only more recently received increased attention through the expanding field of glycobiology. Even though it is of great interest to find reliable, potent and easily available purification methods for galectin-3, the quest has been mostly unsuccessful. Nevertheless, finding quick and efficient method is of interest, but the search could benefit from looking past galectin-3. Cloning, expression and purification of recombinant human galectin-3 is done using BL21 as a host system with His-tag for easier purification using Nickel chromatography, further protein was purified to homogeneity and was confirmed as galectin-3, using SDS-PAGE.

CONSENT

It is not applicable.
ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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