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Expression, purification and characterization of galectin-1 in *Escherichia coli*

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ABSTRACT

As a member of beta-galactoside-binding proteins family, Galectin-1 (Gal-1) contains a single carbohydrate recognition domain, by means of which it can bind glycans both as a monomer and as a homodimer. Gal-1 is implicated in modulating cell–cell and cell–matrix interactions and may act as an autocrine negative growth factor that regulates cell proliferation. Besides, it can also suppress T_H1 and T_H17 cells by regulating dendritic cell differentiation or suppress inflammation via IL-10 and IL-27. In the present study, Gal-1 monomer and concatemer (Gal-1②), which can resemble Gal-1 homodimer, were expressed in *Escherichia coli* and their bioactivities were analyzed. The results of this indicate that both Gal-1 and Gal-1② were expressed in *E. coli* in soluble forms with a purity of over 95% after purifying with ion-exchange chromatography. Clearly, both Gal-1 and Gal-1② can effectively promote erythrocyte agglutination in hemagglutinating activity assays and inhibit Jurkat cell proliferation in MTT assays. All these data demonstrate that bacterially-expressed Gal-1 and Gal-1② have activities similar to those of wild type human Gal-1 whereas the bioactivity of concatemer Gal-1② was stronger than those of the bacterially-expressed and wild type human Gal.

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Introduction

As a member of β-galactoside-binding protein family implicated in modulating cell–cell and cell–matrix interactions [1,2], galectins are composed of β-galactoside binding lectins containing homologous carbohydrate recognition domains (CRDs), which are highly conserved amino acid sequences in the 14 galectin family members that have been identified in mammals thus far [3]. According to their structural features, galectins have been classified into proto-, chimera- and tandem-repeat types [4]. Prototype galectins (Galectins-1, -2, -5, -7, -10, -11, -13, -14 and -15) contain one CRD domain, and usually form homodimers of non-covalently linked subunits [5]. In contrast, in addition to one CRD domain, chimera-type galectins (Galectins-3) have a non-carbohydrate-binding domain [6]. Tandem-repeat type galectins (Galectins-4, -6, -8, -9 and -12) are comprised of two different CRDs joined by a linker peptide and can cross-link glycoproteins due to the presence of more than one CRDs [5]. Galectins of all the three types have hemagglutinating activities, which are attributable to their bivalent carbohydrate-binding properties [7].

Galectin-1 (Gal-1),[3] a 135-amino acid protein in humans, is encoded by the *LGALS1* gene which contains four exons [8,9]. It is ubiquitously distributed in the nucleus, cytoplasm, cell surface and the extracellular space. Although it doesn't have a traditional signal peptide sequence, it is still secreted across the plasma membrane by one or more unidentified, non-classical and secretory pathways [10]. Gal-1 appears both in the form of a monomer and a non-covalent homodimer that contains a single CRD domain, by means of which, Gal-1 can bind glycans. These two forms of Gal-1 are interconverted as homodimers disassociating spontaneously at low concentration [11].

Gal-1 may act as an autocrine negative growth factor that regulates proinflammatory lymphocyte adhesion, migration, polarization, proliferation, apoptosis and differentiation [12–14]. Accumulating evidence has shown that Gal-1 exerts its anti-inflammatory effect by inducing and differentiating $CD4^+Foxp3^+$

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[3] *Abbreviations used:* Gal-1, galectin-1; CRD, carbohydrate recognition domain; ConA, Concanavalin A; LB, Luria–Bertani; IPTG, isopropyl β-D-1-thiogalactopyranoside.

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regulatory T cells (Treg) and $CD4^+IL\text{-}10^+$ T cells (Tr1) [15,16], producing more anti-inflammatory cytokines interleukin (IL)-10 and IL-27 [12,17,18], and inducing innate immunce cells such as dendritic cells and macrophages towards a tolerance phenotype that suppresses differentiation of T helper 1 (T_H1) and T helper 17 (T_H17) cells [19–21]. As a result, Gal-1 is critically involved in various pathological states, including autoimmune diseases and tumor metastasis [22–25], and owing to its critical involvement in the states, recombinant Gal-1 has been used to control various autoimmune and chronic immune-pathological disorders [26,27]. Panjwani and his colleagues found that application of recombinant Gal-1 (rGal-1) after ocular *Pseudomonas aeruginosa* infection significantly ameliorated corneal lesion severity by shifting pro-inflammatory T_H17-mediated pathology toward anti-inflammatory T_H2- and Tr1-type immune responses [20]. Liu et al. reported that recombinant Gal-1 has an anti-viral activity which is capable of reducing LCDV pathogenicity [4].

Although recombinant Gal-1 has been expressed in different systems and demonstrated to have potential anti-inflammatory activity, expression and identification of Gal-1 tandem repeats remain poorly characterized. Given that dimerization of prototype galectin is usually essential for the crosslinking of glycans and subsequent cellular signaling, the aim of this study was therefore to clone and identify the human Gal-1 monomer and concatemer (Gal-1②), which can mimic homodimer of Gal-1, and to compare the bioactivity of these recombinant Gal-1 proteins as well.

Materials and methods

Reagents

Restriction enzymes, Phusion High-Fidelity DNA polymerase and buffers used for cloning were purchased from New England Biolabs (Ipswich, MA). T4 DNA ligase was from TaKaRa (Dalian, China). Oligonucleotides primers were synthesized by BioAsia (Beijing, China). The bacterial expression vector pET-22b and *Escherichia coli* strain BL21 (DE3) were purchased from Novagen (San Diego, CA). Isopropyl β-D-1-thiogalactopyranoside (IPTG) and Concanavalin A (ConA) were from Sigma–Aldrich (St. Louis, MO). Q-Sepharose Fast Flow and SP-Sepharose Fast Flow resins were purchased from GE Healthcare Life Sciences (Uppsala, Sweden). Mouse anti-human Gal-1 monoclonal antibody and HRP-conjugated rabbit anti-mouse IgG were from Abcam (UK, Cambridge). All other reagents were of analytical grade.

Construction of recombinant plasmids pET-22b-Gal-1 and pET-22b-Gal-1②

We have previously amplified *gal-1* gene from human cDNA library with primers 1 and 2 listed in Table 1 and cloned it into pACT plasmid between *Bam*H I and *Kpn* I restriction enzyme sites. DNA sequencing confirmed that the gene was correct (NCBI reference sequence: NM_002305.3) and the plasmid was named "pACTG". For the construction of Gal-1 prokaryotic expression vector, the *gal-1* gene was amplified from the pACTG plasmid with primers 3 and 4 (with *Nde* I and *Sal* I restriction sites respectively introduced and underlined). The PCR products were digested with *Nde* I and *Sal* I restriction enzymes, recovered from agarose gel and cloned into pET-22b(+) plasmid. pET-22b(+) contains a strong T7 promoter in front of multiple cloning sites (MCS) and thereby allows high level expression of recombinant genes located downstream. For the construction of Gal-1② expression vector, one copy of *gal-1* gene was amplified from pACTG plasmid with primers 3 and 5 and *Nde* I and *Bam*H I enzymes recognition sites were added to the products. Another copy was obtained with primers 4 and 6 and *Bam*H I and *Sal* I enzyme recognition sites added. The two PCR products were digested and cloned into pET-22b(+) plasmid between *Nde* I and *Sal* I sites. All the recombinant plasmids were transformed into *E. coli* DH5α competent cells and plasmids were extracted and identified by DNA sequencing. The resulting correct plasmids were designated as "pET-G" and "pET-G②". Finally *E. coli* strain BL21 (DE3), which can produce T7 RNA polymerase once induced by isopropyl β-D-thiogalactopyranoside (IPTG), was transformed with pET-G and pET-G② for recombinant gene expression.

Table 1
Nucleotide sequences of primers used for recombinant Gal-1 gene amplification.

Primer number	Nucleotide sequence
1	5′-CGGGATCCATGGCTTGTGGTCTGGTCG-3′
2	5′-GGGGTACCTCAGTCAAAGGCCACACATTTG-3′
3	5′-GGGAATTCCATATGGCTTGTGGTCTGGTCGCC-3′
4	5′-ACGCGTCGACTCAGTCAAAGGCCACACATTTGATCTTG-3′
5	5′-CGCGGATCCGTCAAAGGCCACACATTTGATCTTGAAG-3′
6	5′-CGCGGATCCATGGCTTGTGGTCTGGTCGCC-3′

Expression of Gal-1 monomer and Gal-1② concatemer

A single colony was selected from pET-G and pET-G② transformed plates respectively and inoculated in 5 ml of Luria–Bertani (LB) medium supplemented with 100 μg/ml of ampicillin and grown at 37 °C with 200 rpm shaking overnight. The cultures were then transferred into 200 ml fresh medium in a shake flask. Protein expression was induced using 1 mM IPTG when the OD_{600} of the culture reached 0.6. Cells were harvested at 12,000 rpm for 20 min after 4 h of induction and the pellet was used for purification. One milliliter of the culture was collected and the pellet was resuspended in 100 μl ddH_2O, mixed with 5 × SDS loading buffer, and heated at 95 °C for 10 min. The sample was centrifuged at 12,000 rpm for 6 min and 10 μl supernatant was analyzed by SDS–PAGE. The uninduced sample was used as negative control.

Fermentation was performed using a 5-L stirred Biostat® bioreactor as previously described [28]. In brief, a single colony was inoculated into 10 ml of LB medium and cultured at 37 °C with shaking overnight. After that, 200 ml of semi-defined medium supplemented with ampicillin was inoculated with the overnight culture and grown at 37 °C until the OD_{600} reached 2–3. Finally, 5 L sterile media (5 g/L Tryptone, 5 g/L yeast extract, 10 g/L glycerol, 2 g/L KH_2PO_4, 4 g/L K_2HPO_4, and 3 g/L $MgSO_4$) were aseptically added to the fermentor and inoculated with the culture as described above. Fermentation was performed using the following parameters: 37 °C, pH 7.0, airflow 5 L pm (1 vvm), agitation 250–800 rpm, and dissolved oxygen 30%. When the OD_{600} reached 10.0, the cultures were induced by addition of IPTG (final concentration is 3 mM) for 4 h. Cells were harvested by centrifugation at 15,000 rpm for 25 min at 4 °C.

Purification of Gal-1 and Gal-1②

To purify recombinant Gal-1 and Gal-1②, 10 g wet weight of cell pellets of BL21 (DE3)/pET-22b-Gal-1 or BL21 (DE3)/pET-22b-Gal-1② was suspended in 100 ml of lysis buffer (20 mM Tris–HCl pH 8.0, 100 mM NaCl, 1 mM EDTA) in a beaker on ice. The cells were disrupted by sonication and the supernatant was collected by centrifugation at 12,000 rpm for 20 min at 4 °C. Then, the supernatant was thoroughly dialyzed against 30 times volume of buffer A (20 mM Tris–HCl pH 7.4, 1 mM EDTA) for 24 h and the insoluble particles were removed by filtering through a 0.22 μm syringe filter (VWR, West Chester, PA). A Q-Sepharose Fast Flow 26 × 200 mm column with 20 ml of column volume (CV) was

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