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Characterization of a Novel Galactose-Binding Lectin Homologue from *Tenebrio molitor*

Ji Eun Jeong, Dong Hyun Kim, Bharat Bhusan Patnaik, Se Won Kang, HeeJu Hwang, Yong Hun Jo, Dae-Hyun Seog, Yeon Soo Han, Yong Seok Lee

***Abstract*—**An expressed sequence tag (EST) analysis provideus portions of expressed genes. We have constructed cDNA library and determined randomly sequences from cDNA library clones of *T. molitor* injected with acholeplasma lysate. We identified the homologous to a galectin gene. As the result of cloning and characterization of novel, we found that the protein has an open reading frame (ORF) of 495 bp, with 164 amino acid residues and molecular weight of 18.5 kDa. To characterize the role of novel Tm-galectin in immune system, we quantified the mRNA level of galectin at different times after treatment with immune elicitors. The galectin mRNA was up-regulated about 7-folds within 18 hrs. This suggests that Tm-galectin is a novel member of animal lectins, and has a role in the process of pathogen recognition. Our study would be helpful for the study on immune defense system and signaling cascade.

***Keywords*—**EST, Innate immunity, *Tenebrio molitor*, Galectin.

I. Introduction

TENEBRIO MOLITOR has been used as a model system to study innate immune responses against Gram +/- bacteria containing peptidoglycan. Immune system of insect has been developed to defend against various pathogen invasions and their immune responses have been described as including humoral and cellular reaction. A pattern recognition proteins (PRPs) are well known as components of the invertebrate immune system including hemolymph coagulation and melanization [1].

Galectin is known to bind β-galactoside sugars and has functions on the mediation of cell-cell interactions, cell-matrix adhesion and transmembrane signaling [2], [3]. Also, it is often involved in several diseases such as cancer, HIV, autoimmune disease, chronic inflammation [4], graft vs. host disease (GVHD) and allergic reactions. It is thought to be targets for the development of new anti-inflammatory and anticancer therapies [5].

Lectins bind to microbial pathogens and consist of saccharide found on microbial surface glycoproteins such as β-1, 3-glucans, lipopolysaccharides (LPS) and peptidoglycan [6], [7].The important role of lectins has been known to recognize carbohydrates that are found on pathogens, or that are inaccessible on host cellsin the defense system [8].

Recently, many researchers have attempted antimicrobial defense systems using large beetles [9]. Here, we attempted to completely characterize the novel galectin homologue from the beetle. This gene has been reported to be up-regulated after immune elicitation and its possible involvement in innate immune defense pathway.

J.E. Jeong is research professor in Soonchunhyang University, Asan-si, Korea.
D.H. Kim is a Ph. D. candidate in Chonnam National University, Gwangju, Korea.
B. B. Patnaik is working of the College of Agriculture and Life Science, Chonnam National University, Gwangju, Korea.
S.W. Kang is a Ph. D. candidate in Inje University, Busan, Korea.
H.J. Hwang is researching in Soonchunhyang University, Asan-si, Korea.
Y.H. Jo is working of the College of Pharmacy, Pusan National University, Busan, Korea.
D. H. Seog is a professor of the Department of Biochemistry, College of Medicine, Inje University,Busan, Korea.
Y. S. Han, corresponding author, is a professor of the College of Agriculture and Life Science, Chonnam National University, Gwangju, Korea (e-mail: hanys@chonnam.ac.kr).
Y.S. Lee, another corresponding author, is a professor of the Department of Life Science and Biotechnology, College of Natural Sciences, Soonchunhyang University, Asan-si, Korea (e-mail:yslee@sch.ac.kr).

II. Material and Method

A. Sample Preparation

Larvae of mealworm beetle, *T. molitor* were reared on wheat bran meal in an environmental chamber at 25 ± 1°C with 60 ± 5% relative humidity and a 16:8 hr light and dark cycle.

B. cDNA Library Construction

We used a cDNA library construction using SMART cDNA Library Construction Kit (Clontech).Randomly selected 2000 clones were sequenced using Applied Biosystems Sequencer.

The full-length cDNA of Tm-galectin gene, PCR- based screening of cDNA library was conducted using forward and reverse primer. The gene products were purified using gel extraction kit (Gene All, Korea). The PCR products were consequently ligated into TOPO TA cloning kit (with pCR2.1-TOPO cloning vector) and transformed into TOP10 chemically competent *E. coli* (Invitrogen Corporation, Carlsbad, CA) and sequenced using primer M13-F and M13-R.

C. DNA Sequence Analysis

The cDNA and deduced amino acid sequence of Tm-galectin was analyzed using UltraEdit-32 software package. The deduced amino acid sequence was predicted by ORF finder online in NCBI (www.ncbi.nih.gov) and the Expert Protein Analysis System (http://www.expasy.org/). The protein domains were predicted with the simple modular architecture research tool (SMART) version 4.0 (http://smart.embl-heidelberg.de/). Multiple sequence alignment was performed using the ClustalX (version 1.83) program. The presumed secondary structure of Tm-galectin was established using the

SWISS-MODEL workspace (http://swissmodel.expasy.org/) and PSIPRED secondary structure prediction method (bioinf.cs.ucl.ac.uk/psipred).

D. Gene Expression Analysis by Quantitative Real-Time PCR

Expression of galectin mRNA was examined after *T. molitor* larvae were injected by immune elicitors. Third instar larvae were injected with 1 $\mu\ell$ of *Acholeplasma* cell lysates, using Nano injector (Drummond Scientific Co., Broomall, PA, USA). The control group was injected only with injection buffer. Total RNA from the larvae were collected 3, 6, 12, 18 and 24h after injection. 2µg of total RNA was reverse-transcribed in a 50-µl reaction mixture with a High capacity cDNA Reverse Transcription Kit (Bioneer, Korea). qRT-PCR was performed on Exicycler™ 96 Real-Time Quantitative Thermal Block (Bioneer, Korea).

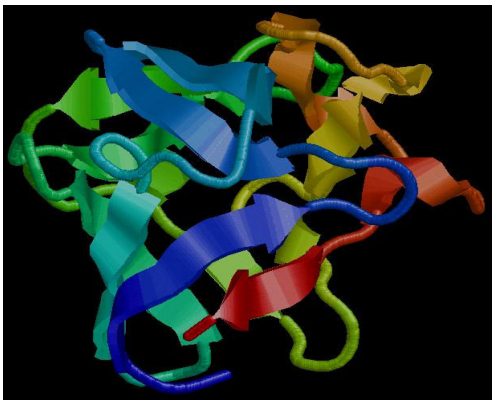
III. Result and Discussion

We identified a single EST, homologous to the characterized galectin genes by sequencing of clones from *T. molitor* cDNA library treated with *Acholeplasma* lysate. To characterize Tm-galectin full-length cDNA, the clones corresponding to the EST were re-sequenced and yielded cDNA sequence of 495bp (Fig. 1). The Tm-galectin cDNA encodes a polypeptide of 164 amino acids with a theoretical isoelectric point of 8.69 and predicted molecular weight of 18.5kDa. Predictions from ScanProsite analysis showed that the amino acids from 67 (Q) to 162 (T) belonged to a Carbohydrate recognition domain (CRD) formed from presumed gene triplication (Ricin-type beta tre-foil). Signal 4.0 server results didn't predict any signal peptide sequence corresponding to a classical secretion identity or a transmembrane domain, characteristic of galectin family members.

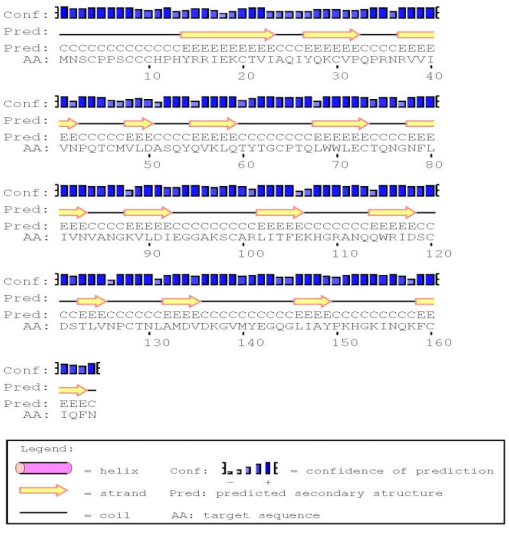
Secondary structure prediction was conducted using the PSIPRED software tool that showed that Tm-galectin was primarily composed of sheets and coils with absence of helical regions (Fig. 2 (a)). Swiss-model predictions of Tm-galectin structure based on homology modeling presumed it to be a jelly roll or a manifold-curve β-hairpin (Fig. 2 (b)).

To characterize the role of novel Tm-galectin in immune system, we quantified the mRNA level of galectin at different times after challenge to the larvae with immune elicitors. The expression of galectin in control (uninjected) and immune elicitor challenged *T. molitor* larvae were examined with qPCR. The greatest change of transcript expression in larvae challenged with *Acholeplasma* lysate was observed 18 hrs (Fig. 3). In this case, the galectin mRNA was up-regulated about 7-folds within 18 hrs.

Fig. 1 Nucleotide and deduced amino acid sequence of cDNA encoding putative galectin homologue



(a)



(b)

Fig. 2 Secondary structure of Tm-galectin predicted using the PSIPRED software tool (a) and Swiss-model (b)

Though the response time and the pattern of recognition were not varied to the immune elicitors, it is suggested that the novel galectin homologue is a capable recognition protein and would play a definitive function in the immune defense against invaders. The role of putative Anopheles galectin as a PRR by binding saccharide ligands on the microbial surface to trigger a host immune response is known with the upregulation observed

in the salivary glands and gut of Anopheles mosquito infected with malaria or bacteria [10].

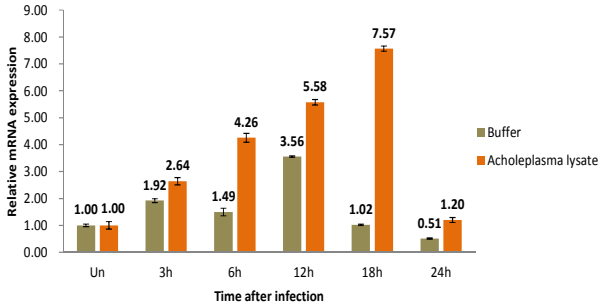


Fig. 3 Quantitative analysis of galectin mRNA at different times after injection of acholeplasma lysate

IV. Conclusion

Tenebrio molitor has been used as a good model for studies in biochemistry, immunology and physiology. We characterized that novel galectin homologue from *T. molitor* is the first galectin reported by sequencing of clones from cDNA library treated with *Acholeplasma* lysate. Though their function in host defense system has to be studied more detail, it will be interesting to study, the remarkable evolutionary plasticity shown by the pathogens and parasites in evading the galectins by mimicking their hosts to gain entry into the hosts. Also, transcriptomic database construction will be useful to study defense mechanism of innate immunity.

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