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ScienceDirect
Gene 411 (2008) 46–58
GENE
www.elsevier.com/locate/gene

Molecular and functional characterization of a tandem-repeat galectin from the freshwater snail *Biomphalaria glabrata*, intermediate host of the human blood fluke *Schistosoma mansoni*

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Received 11 August 2007; received in revised form 19 December 2007; accepted 7 January 2008
Available online 17 January 2008
Received by F.G. Alvarez-Valin

Abstract

In the present study, a tandem-repeat type galectin was characterized from an embryonic cell line (Bge) and circulating hemocytes of the snail *Biomphalaria glabrata*, intermediate host of the human blood fluke *Schistosoma mansoni*. The predicted *B. glabrata* galectin (BgGal) protein of 32 kDa possessed 2 carbohydrate recognition domains, each displaying 6 of 8 conserved amino acids involved in galactoside-binding activity. A recombinant BgGal (rBgGal) demonstrated hemagglutinating activity against rabbit erythrocytes, which was specifically inhibited by galactose-containing sugars (lacNAc/lac > galNAc/gal). Although native galectin was immunolocalized in the cytoplasm of Bge cells and the plasma membrane of a subset of snail hemocytes (60%), it was not detected in cell-free plasma by Western blot analysis. The findings that rBgGal selectively recognizes the schistosome-related sugar, lacNAc, and strongly binds to hemocytes and the tegument of *S. mansoni* sporocysts in a sugar-inhibitable fashion suggest that hemocyte-bound galectin may be serving as a pattern recognition receptor for this, or other pathogens possessing appropriate sugar ligands. Based on molecular and functional features, BgGal represents an authentic galectin, the first to be fully characterized in the medically-important molluscan Class Gastropoda.

Keywords: Lectin; Gastropod mollusk; Hemocyte; Bge cell line; Innate immunity

1. Introduction

Galectins represent a large family of structurally-related, phylogenetically diverse lectins with a carbohydrate binding specificity primarily to ß-galactoside residues. In mammalian species this family is currently represented by 15 members (Gal-1 through-15) that are differentiated on the basis of the number of carbohydrate recognition domains (CRDs), presence/length of a CRD linker peptide or N-/C-terminal tails, amino acid sequence homology, especially of highly conserved residues within the CRDs, and its metal ion-independent functionality (Barondes et al., 1994; Cooper, 2002; Leffler et al., 2004). Despite their narrow ligand-binding affinity for ß-galactosides, galectins have been implicated in a diversity of cellular functions including cell adhesion/proliferation, development/morphogenesis, tumor cell metastasis and immune regulation/innate immunity (Hughes, 2001; Vasta et al., 2004a; Zick et al., 2004; Camby et al., 2006). The role of galectins as effectors or modulators of the immune response has been most extensively studied in vertebrates where they have been implicated in

Abbreviations: BgGal, *Biomphalaria glabrata* galectin; gal, galactose; galNAc, *N*-acetyl-galactosamine; lac, lactose; lacNAc, *N*-acetyl-lactosamine; glcNAc, *N*-acetyl-glucosamine; man, mannose; glc, glucose; tre, trehalose; LDN, lacdiNAc; Bge, *Biomphalaria glabrata* embryonic; CRD, carbohydrate recognition domain; His, histidine; RBC, red blood cell; DIC, differential interference contrast; PRR, pattern recognition receptor; aa, amino acid; EDTA, ethylenediamine-tetra acetic acid; EST, expressed sequence tag; cDNA, complementary DNA; RACE, rapid amplification of cDNA ends; FBS, fetal bovine serum.

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doi:10.1016/j.gene.2008.01.003

apoptotic regulation of B/T-cell populations, cytokine signaling, monocyte/macrophage-mediate inflammation and microbe phagocytosis (Sano et al., 2003; Young and Meeusen, 2004; Acosta-Rodriguez et al., 2004; Liu, 2005; Rabinovich and Gruppi, 2005; Rubinstein et al., 2006; Barrionuevo et al., 2007).

Metazoan invertebrates representing a diversity of major phyla also possess multiple members of the galectin superfamily as evidenced by both molecular and functional criteria (Vasta et al., 2004b). These are most highly represented in such organisms as nematodes (Hirabayshi et al., 1992; Greenhalgh et al. 1999; Newlands et al., 1999), arthropods (Pace et al., 2002; Pace and Baum, 2004, Barat-Houari et al., 2006; Huang et al., 2007), tunicates (Parrinello et al., 2007) and sponges (Pfeifer et al., 1993; Stalz et al., 2006). In the Phylum Mollusca, the presence of galactose-binding lectins also has been demonstrated (e.g., Suzuki and Mori, 1989; Mitra and Sarkar, 1998; Wilson et al., 1992; Ozeki, 1998), and in some species, the molecular mass of isolated lectins were consistent with galectins possessing single (Mitra and Sarkar, 1998) or dual (Ozeki, 1998) CRDs. In addition, expressed sequence tags (EST)/partial sequences for galectin homologues have been identified (Rafferty and Powell, 2002; Mitta et al., 2005; GenBank™ accession nos. AJ550634, BG467428, CO635934, CX6376, EE722624, CK989149, CN476116), including a complete coding sequence from the abalone *Haliotis* (GenBank™ accession no. EF392832). Thus, there is a substantial support for the existence of this gene family in molluscs. However, although galactose-binding proteins previously have been reported in the hemolymph of bivalve (Suzuki and Mori, 1989; Baldo and Uhlenbruck, 1975), gastropod (Mitra and Sarkar, 1998; Mansour, 1996), and cephalopod (Rogener et al., 1985) molluscs, their molecular structures, expression profiles and specific role(s) in the internal defense system of these organisms remain unknown.

Despite evidence for galectin-like proteins within the molluscs, detailed studies characterizing the structure, ligand-binding properties and protein expression of galectins has been very limited in this animal group. To date only one other molluscan galectin, that of the oyster *Crassostrea virginica* has been characterized both functionally and at the molecular level (Tasumi and Vasta, 2007). In the present study, we report the cloning and functional characterization of a tandem-repeat type galectin from circulating phagocytic hemocytes of the freshwater snail *Biomphalaria glabrata*, and demonstrate its binding reactivity with the tegumental surface of larval *Schistosoma mansoni*, a human blood fluke that utilizes *B. glabrata* as its intermediate host. To our knowledge this study represents the first investigation of a galectin at the molecular level from a mollusc representing the medically-important Class Gastropoda.

2. Materials and methods

2.1. Cell and tissue sources used in the study

Cultures of the *B. glabrata* embryonic (Bge) cell line were obtained from American Type Culture Collection (ATCC CRL 1494; Rockville, MD) and maintained in 50 cc culture flasks in complete Bge medium (Hansen, 1976) containing heat-inactivated 10% fetal bovine serum (FBS), penicillin and streptomycin, at 26 °C under atmospheric conditions (Yoshino and Laursen, 1995). Whole hemolymph, containing circulating hemocytes, was obtained from lab-reared *B. glabrata* snails (BS-90 strain) as detailed in Section 2.7. Snails were maintained in 10-gal aquaria at 26 °C and fed leaf lettuce ad libitum.

2.2. RNA extraction and rapid 5′ and 3′ amplification of cDNA ends (RACE)

Bge cells and *B. glabrata* hemocytes were isolated as previously described (Humphries and Yoshino, 2006). Total RNA from both cell sources was extracted with Trizol (Invitrogen Corporation, Carlsbad, CA), followed by precipitation with isopropanol according to the manufacturer's protocol and stored at −80 °C until further use. An EST (GenBank™ accession no. AW740392) from a BS90 *B. glabrata* hemocyte library was selected for its high similarities to Gal-4. In order to obtain the complete coding sequence several primers, [forward (65: 5′ TCAATCACCGCATTCACCC 3′; 92: 5′ GTGTGTCTCACT TGAACATCC 3′) and reverse (189: 5′ TGGTCTATTGTCC GCTGCTG 3′; 134: 5′ GACTCAAGTTGACATCACCC 3′)], were designed and used in RACE reactions (First Choice™ RLM-RACE Kit, Ambion, Applied Biosystems Business, Austin, TX) to amplify both 5′- and 3′-ends thus permitting the completion and cloning of the full-length cDNA sequence from Bge cells.

2.3. Cloning and sequencing

Cloning of the complete cDNA sequence was performed following previously described protocols (Dinguirard and Yoshino, 2006). Briefly, specific primers (Fw: 5′: ATGGCAT ATCCTGTACCTTACTC 3′; Rv: 5′ TTGGTCTATTGTCCG CTGC 3′) were used to amplify the desired 900 bp galectin sequence from Bge cell and hemocyte template cDNA. The amplified product was then purified, cloned and sequenced using the plasmid-based primers T7 (5′GGCCGCG-GGAATTCGATT 3′) and sp6 (5′GATTTAGGTGACACTA-TAG 3′). Samples were sequenced at the Biotechnology Center (University of Wisconsin-Madison) using an Applied Biosystems 3730_{XL} automated DNA analyzer (Foster City, CA) incorporating 50 cm capillary arrays on a POP-7 matrix. Data were analyzed using PE-Biosystems Sequencing Analysis software, version 3.7.

2.4. Sequence analysis and bioinformatics procedures

Complementary DNA sequence identity and homology analyses were performed using the Basic Local Alignment Search Tool (BLAST-X) from the National Center for Biotechnology Information (NCBI) database. The predicted amino acid determinations, sequence alignment analyses, presence/absence of hydrophobic domains using the Kyte–Doolittle method, and cluster tree analysis were performed using Vector NTI 8 (InforMax, Inc., Invitrogen). Testing for the presence of signal