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Purification and characterization of a T-antigen specific lectin from the coelomic fluid of a marine invertebrate, sea cucumber (*Holothuria scabra*)

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Abstract A novel lectin was purified from the coelomic fluid of the sea cucumber *Holothuria scabra* (HSL), subjected to bacterial challenge. HSL is a monomeric glycoprotein of molecular mass 182 kDa. The lectin is highly thermostable as it retains full activity for 1 h at 80 °C. Further, the hemagglutination activity of HSL is unaffected by pH in the range 2–11. Unlike other lectins purified from marine invertebrates, the hemagglutination activity of HSL does not require any divalent metal ions. The affinity profile of HSL was studied by a combination of hemagglutination inhibition and fluorescence spectroscopy. HSL binds to desialylated glycoproteins, MeαGal, T-antigen and T (α-ser)-antigen with a distinction between β1–4 and β1–3 linkages. Meα-T-antigen was a potent ligand having highest affinity (K_a 8.32 $\times$ 10^7 M^{-1}). Monosaccharide binding is enthalphically driven while disaccharide binding involves both entropic and enthalpic contributions.

Introduction

Lectins are mono- or multivalent, proteins or glycoproteins of non-immunogenic origin, which recognize specific carbohydrate structures reversibly in a non-catalytic manner [1]. They are ubiquitous in nature, and are found in plants, microorganisms and animals including marine invertebrates. In marine invertebrates, lectins have been suggested to participate in innate immune response by inducing bacterial agglutination or by acting as opsonins to enhance phagocytosis by coelomocytes [2–5]. Besides roles in cell recognition and host defense, lectins have long been used as probes to determine sugar composition

Abbreviations: HSL, *Holothuria scabra* lectin; 2dGal, 2-deoxy-D-galactose; Me α/β Gal, methyl α/β-D-galactopyranoside; T-antigen, Gal β1–3 GalNAc; T (α-ser)-antigen (Gal β1–3 GalNAc α-1-*O*-L-Ser), Thomsen–Friedenreich antigen; Meα-T-antigen, Gal β1–3 GalNAc α-1-*O*-Me; Meβ-T-antigen, Gal β1–3 GalNAc β-1-*O*-Me.

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of glycan and glycoconjugates like bacterial lipopolysaccharide, cell surface glycoproteins and glycolipids [6]. Therefore, it is essential to understand the mechanism of ligand binding to lectin, in order to facilitate their use as an analytical tool and for better characterization of lectin interaction with cell bound carbohydrates.

A large number of lectins have been isolated and characterized from marine sources, some of them are from sponges [7–10], tunicates [11–14], crustaceans [15–17], and molluscs [18]. However very few lectins are reported in echinoderms in comparison with those of other marine invertebrates. These include a few lectins from sea urchin, a sialic acid binding lectin belonging to the heat shock protein family [19], endogenous echinonectin from *Lytechinus variegates* [20], Lectin from *Asterina pectinifera* [21] and evidence for the involvement of lectin in the complement homologous immune system [4]. Sea cucumbers are echinoderms inhabitating relatively shallow coastal areas experiencing little or no currents. Some lectins have been isolated from different species of sea cucumbers, including four Ca^{2+} dependent, galactose/GalNAc specific (CEL I, II, III and IV) lectins from *Cucumaria echinata* [22]; a mannan-binding lectin from coelomic fluid of *Cucumaria japonica* [23]; four structurally distinct Ca^{2+} dependent lectins; uronic acid (SPL-1), GalNAc (SPL-2, SJL-I) and lactose/melibiose (SJL-II) specific lectins from *Stichopus japonicus* [24,25]; and hemagglutinins and hemolysins from coelomic fluid of *Holothuria polii* [26,27]. However, none of the marine invertebrate lectins have been explored for their sugar-binding kinetics and thermodynamics in detail. In this article, we report our extensive studies on isolation and characterization of a lectin, named HSL, from the coelomic fluid of a sea cucumber, *Holothuria scabra*. The specificity profile and thermodynamic properties of HSL were studied by a combination of hemagglutination inhibition and fluorescence spectroscopy.

Materials and methods

Materials

D-glucose, D-mannose, D-galactose, D-galactosamine, 2-deoxy-D-galactose, L-fucose, methyl α-D-galactose, methyl β-D-galactose, D-maltose, α-lactose, α-D-melibiose, raffinose, stachyose, phenyl-sepharose CL-4B, pronase-E, carboxypeptidase, aminopeptidase, fetuin (bovine), fibrinogen (human), thyroglobulin (bovine), were obtained from Sigma chemical Co. St. Louis, USA; molecular weight markers and Sephacryl S-300 from Amersham Biosciences, Sweden; holotransferrin (bovine) was from Calbiochem, CA, USA; while Gal α1–3 Gal, Gal α1–4 Gal, Gal β1–4 GlcNAc, Gal β1–3 GlcNAc, Gal β1–4 GlcNAc, Gal β1–3 GalNAc, Gal β1–3 GalNAc α 1-*O*-L-Serine, Gal β1–3 GalNAc α 1-*O*-methyl and Gal β1–3 GalNAc β 1-*O*-methyl were purchased from Dextra Labs, London, UK. All other chemicals used were of analytical grade.

Purification of lectin

Adult sea cucumbers (*Holothuria scabra*) were collected from Anjuna coast of Goa, India and maintained in running seawater facility at National Institute of Oceanography, Goa. Lectin accumulation was induced by challenging about 5–6 animals with a cocktail of six different bacterial strains that include, *Staphylococcus citreus*, Group D *Streptococci*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia morganii* and *Shigella* sp. Coelomic fluid was collected from the challenged animal on 5th day, in 1:1 ratio with Alsever's solution as anticoagluant (dextrose 20.5 g, trisodium citrate 8 g, citric acid 0.55 g and sodium chloride 4.2 g in 1000 mL water). The resulting solution was centrifuged ($5000 \times g$, 15 min at 4 °C) to remove coelomocytes and the clear supernatant was treated with serine proteinase inhibitor (PMSF, 20 μg mL^{-1}) and stored at −20 °C.

Coelomic fluid preparation was concentrated over a 10 kDa ultra filtration membrane (to remove <10 kDa contaminants) and heat treated at 60 °C for 2 h to inactivate proteases. Tris–HCl buffer, pH 8.5 (TB) was added to a final concentration of 20 mM. Solid ammonium sulphate was added to the preparation to a final concentration of 3% (w/v). The sample was then passed through hydrophobic interaction column (phenyl sepharose), pre-equilibrated with TB containing 3% (w/v) ammonium sulphate. The column was washed with loading buffer until A_{280} of the eluate returned to baseline. The bound lectin was eluted with TB. Fractions showing hemagglutination activity were pooled together, treated with ammonium sulphate to a final concentration of 1% (w/v) and re-loaded on phenyl sepharose column, pre-equilibrated with TB containing 1% (w/v) ammonium sulphate. Bound protein was eluted with TB. Fractions with lectin activity were pooled together and stored at 4 °C until further use.

Protein concentration was determined according to the method of Bradford [28] using bovine serum albumin as standard. Total neutral sugar content of the lectin was estimated by phenol-sulphuric acid method of Dubois et al. [29], using galactose-mannose (4:3) as standard.

Erythrocyte preparation, hemagglutination and inhibition assays

Human erythrocytes of blood group A, B and O were washed 4–5 times with 20 mM Tris–HCl buffer pH 7.2 containing 150 mM NaCl (TBS) and a final suspension of 3% (v/v) was prepared in TBS and treated with different enzymes, pronase (0.5 mg mL^{-1}), trypsin (2.5 U mL^{-1}) and neuraminidase (0.1 U mL^{-1}) at 37 °C for 1 h. Enzyme treated erythrocytes were washed four times with TBS before use.

Hemagglutination tests were performed in standard microtitre plates with U-bottom wells by two-fold serial dilution method. A 50 μl aliquot of erythrocyte suspension was mixed with 50 μl of serially diluted coelomic fluid, incubated for 1 h and visually examined for agglutination. The unit of activity (HA units) was expressed as the reciprocal of the highest dilution (titre) of the lectin that showed complete agglutination. The specific activity of the lectin is defined as the hemagglutination units per milligram of protein (HA unit mg^{-1}).

Hemagglutination inhibition assays were performed essentially as hemagglutination assay, except that serial dilution of various sugar solutions (25 μL) were pre-incubated for 15 min at room temperature with 25 μL of