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New GlcNAc/GalNAc-specific lectin from the ascidian *Didemnum ternatanum*

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Abstract

Previously we isolated GlcNAc-specific lectin (DTL) from the ascidian *Didemnum ternatanum* by affinity chromatography on cross-linked ovalbumin. Here we report the purification and characterization of new D-GlcNAc/D-GalNAc-specific lectin DTL-A from the same ascidian. This lectin was isolated from non-bound cross-linked ovalbumin fraction and further was purified by gel filtration on Sepharose CL-4B, affinity chromatography on GlcNAc-agarose and gel filtration on Superdex 200. SDS-polyacrylamide gel electrophoresis and gel filtration of purified lectin on Sepharose CL-4B indicates that it exists as large aggregates in the native state. Investigations of the carbohydrate specificity of DTL-A by enzyme-linked lectin assay suggest the multi-specificity of this lectin. DTL-A binds BSM, asialo-BSM as well as heparin and dextran sulfate. The binding of DTL-A to BSM was inhibited by monosaccharides D-GlcNAc and D-GalNAc, their α- but not β-anomers. Among polysaccharides and glycoconjugates, DTL-A binding to BSM was effectively inhibited by BSM, asialo-BSM, pronase-treated BSM and synthetic α-D-GalNAc-PAA. Fetuin and asialofetuin showed a much lower inhibitory potency, heparin and dextran sulfate were noninhibitory. On the other hand, DTL-A binding to heparin was effectively inhibited by dextran sulfate, fucoidan, whereas BSM showed insignificantly inhibitory effect. DTL-A binding to heparin was not inhibited by D-GlcNAc and D-GalNAc.

Keywords: Invertebrate; *Didemnum ternatanum*; GlcNAc/GalNAc-specific lectin; Glycoprotein; Sulfated polysaccharide

1. Introduction

Lectins are multivalent carbohydrate-binding proteins found in plants, bacteria and animals including marine invertebrates. They are used in the purification of polysaccharides and glycoproteins and in a variety of biological applications including cell separation, mitogenic stimulation of immune cells, identification of blood groups and microorganisms, and monitoring alterations on the surface of normal and neoplastic cells [1]. A number of invertebrate lectins have been reported. Although in most cases physiological functions of invertebrate lectins are not completely clear, there are increasing data suggesting the involvement of these lectins in processes of differentiation and development of organisms as well as in the elimination of foreign substances through binding to their carbohydrate structures [2–6]. An investigation of lectins from marine invertebrates will allow a deeper understanding of their biological role. On the other hand, they can be used as valuable tools in biomedical research.

Previously we isolated GlcNAc-specific lectin from ascidian *Didemnum ternatanum* (DTL) [7]. Herein we report the purification and characterization of new GlcNAc/

Abbreviations: DTL-A, *Didemnum ternatanum* lectin; BSM, bovine submaxillary mucin (type 1S); HRP, horseradish peroxidase; BSA, bovine serum albumin; EDTA, ethylenediamine tetraacetate; PAA, polyacrylamide; PBS, phosphate-buffered saline; TBS, tris-buffered saline

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GalNAc-specific lectin from the same ascidian (DTL-A). GlcNAc/GalNAc-specific lectins were isolated previously from many invertebrate species [8–11], but a difference of DTL-A from other lectins with similar carbohydrate specificity was observed in respect to metal and pH dependence, molecular masses and subtle carbohydrate specificity.

2. Materials and methods

2.1. Materials

Monosaccharides were obtained from Merk (Darmstadt, Germany). The disaccharides Gal(β1→4)GlcNAc, Gal(β1→3)GalNAc, *p*-nitrophenyl glycosides, fetuin, asialofetuin, human α_1-acid glycoprotein, BSM (type1S), ovalbumin (grade V), ovomucoid, λ- and χ-carrageenans, chondroitin sulfate A, heparin, pronase E, horseradish peroxidase, trypsin and *N*-acetyl-D-glucosamine-agarose were purchased from Sigma Chemical (USA). Chitooligosaccharides were a gift of Dr. V.I. Gorbach of our institute. Fucoidan from *Fucus vesiculosus* and alginic acid were obtained from Fluka (Switzerland), pectin was from Schuchardt (München). The synthetic PAA-based glycoconjugates were gifts of Dr. N.V. Bovin (M.M. Shemyakin Institute of Bioorganic Chemistry, Moscow). The methyl glycosides of *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine were synthesized by Dr. E. Evtushenko of our institute. Human erythrocytes were obtained as outdate red cell concentrates from the Center of blood utilization (Vladivostok). Sepharose CL-4B, Sephadex G-150, dextran sulfate (mol. mass ~500,000), and dextran T-500 were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Superdex 200 HR 10/30 column, the standard proteins used for apparent molecular mass estimation by SDS-PAGE were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden).

2.2. Isolation and purification of DTL-A

DTL-A was isolated from ascidian *D. ternatanum* collected at the South Region of the Pacific Ocean. The lyophilized ascidians (15 g) were homogenized in 10 volumes (w/v) of 0.9% NaCl. The homogenate was stirred for 16 h at 4 °C and centrifuged at 4000 rpm for 20 min. Crude saline extract (150 ml) was dialyzed for 10 h against 0.01 M PBS, pH 7.4 and centrifuged. The supernatant was applied on cross-linked ovalbumin, previously equilibrated with PBS. The non-bound fraction with cross-linked ovalbumin was concentrated by ultrafiltration and applied on Sepharose CL-4B (2.4×90 cm) equilibrated with 0.1 M PBS, pH 8.0. The column was eluted with the same buffer at 10 ml/h and fractions showing hemagglutinating activity were pooled and applied to GlcNAc-agarose (40 ml). The affinity column was washed with buffer until the baseline. The fraction bound to the sorbent was eluted with 0.1 M PBS, pH 7.4, containing 0.2 M *N*-acetyl-D-glucosamine, dialyzed against water and lyophilized. The GlcNAc-agarose bound fraction was additionally purified by fast protein liquid chromatography on Superdex 200 HR 10/30 column.

2.3. SDS-PAGE and binding assay of DTL-A transferred on nitrocellulose membrane with heparin–HRP

SDS-PAGE was carried out according to the procedure of Laemmli [12] using 15% of polyacrylamide separation gel and 4% polyacrylamide stacking gel. The molecular mass of DTL-A was determined by SDS-PAGE in the presence and absence of 2-mercaptoethanol. The reduction of DTL-A was performed by heating at 100 °C for 5 min in a sample buffer containing 2% SDS and 2.5% 2-mercaptoethanol. Gels were calibrated using the following standard proteins: phosphorylase B (94000), BSA (67000); ovalbumine (45000); carbonic anhydrase (30000); trypsine inhibitor (20100), and alfa-lactalbumine (14400).

The electrophoresed lectin was transferred from the gel to nitrocellulose at 400 mA for 1 h, using a Hoefer TE Series electrophoresis unit and 25 mM Tris, 192 mM glycine, 20% (v/v) methanol as transfer buffer. The reactive bands were detected using heparin conjugated with HRP. The conjugate heparin–HRP was received as described [13]. Prior to conjugation, heparin was modified to increase the number of free amino group [14]. The membrane was developed using substrate 3.3′-diaminobenzidine.

2.4. Hemagglutination assay

To assay the hemagglutinating activity, DTL-A was 2-fold serially diluted with 0.01 M PBS (25 μl) in the microtiter U-plates. To each well, an equal volume of 2% suspension of human erythrocytes was added and the mixture was agitated. The hemagglutination was visually evaluated after 30 min.

For the hemagglutination inhibition assay, the aqueous solutions of various substances were 2-fold serially diluted with PBS. DTL-A (25 μL, 4 doses of agglutination) and 2% erythrocyte suspension (25 μl) were added to each sample (25 μl) successively. The mixture obtained was gently stirred by pipette and kept for 1 h. The minimal concentration of each substance required for complete inhibition was determined.

2.5. Solid phase assay

Microtiter plate wells (Dynatech Laboratories Inc.) were coated with heparin or BSM by incubation overnight at 4 °C with 100 μl of solution in 0.1 M carbonate/bicarbonate buffer (pH 9.5). The wells were washed 3 times with PBS (pH 7.2) containing 0.05%