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L-rhamnose-binding lectin from eggs of the *Echinometra lucunter*: Amino acid sequence and molecular modeling

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ABSTRACT

An L-rhamnose-binding lectin named ELEL was isolated from eggs of the rock boring sea urchin *Echinometra lucunter* by affinity chromatography on lactosyl-agarose. ELEL is a homodimer linked by a disulfide bond with subunits of 11 kDa each. The new lectin was inhibited by saccharides possessing the same configuration of hydroxyl groups at C-2 and C-4, such as L-rhamnose, melibiose, galactose and lactose. The amino acid sequence of ELEL was determined by tandem mass spectrometry. The ELEL subunit has 103 amino acids, including nine cysteine residues involved in four conserved intrachain disulfide bonds and one interchain disulfide bond. The full sequence of ELEL presents conserved motifs commonly found in rhamnose-binding lectins, including YGR, DPC and KYL. A three-dimensional model of ELEL was created, and molecular docking revealed favorable binding energies for interactions between ELEL and rhamnose, melibiose and Gb_3 (Galα1-4Galβ1-4Glcβ1-Cer). Furthermore, ELEL was able to agglutinate Gram-positive bacterial cells, suggesting its ability to recognize pathogens.

1. Introduction

Rhamnose-binding lectins (RBLs) belong to a group of lectins that bind specifically to L-rhamnose and α-galactose, rather than β-galactosides, and do not require divalent cations or thiol groups for their hemagglutinating activity [1,2]. Many RBLs are composed of repeated carbohydrate recognition domains (CRD), containing about 95 amino acid residues each and a unique α/β fold with long structured loops important for monosaccharide recognition [3,4]. These lectins share four conserved disulfide bonds and two conserved motifs: -ANYGR(TD)- in N-terminal and -DPCX(G)T(Y)KY(L)- in C-terminal, which are involved in the primary recognition of ligands [4–6].

The biological function of RBLs is not yet known. However, it is likely that these lectins play a role in the production of reactive oxygen species and phagocytosis, regulation of carbohydrate metabolism, prevention of polyspermy and self-defense [7–9]. Members of the RBL family act as pattern recognition receptors (PRRs), recognizing various pathogen-associated molecular patterns (PAMPs). RBLs can recognize lipopolysaccharides and lipoteichoic acid, constituents of cell wall of Gram-positive and Gram-negative bacteria, respectively, thus acting as opsonins [10–12].

Structurally, RBLs can be divided into five subfamilies. Type I possesses three repeated domains, and Type II is composed of two repeated domains with an extra domain. Type III and Type IV have two repeated domains, but they have different sugar specificity. Finally, Type V has only one domain able to form a homodimer with a disulfide bond between subunits [3].

Most RBLs studied have been found in fish eggs and ovaries. However, the first RBL described, the sea urchin eggs lectin, or SUEL, was purified from eggs of the sea urchin *Anthocidaris crassispina* [13,14]. Also, RBLs have been described in colonial ascidians and

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bivalves [12,15]. Interestingly, SUEL is unique member of the Type V subfamily.

The rock boring sea urchin *Echinometra lucunter* Linnaeus is commonly found throughout the Caribbean Sea and South America coast. It commonly occurs on coral reefs and shallow rock areas where depths reach 2 m. Great populations of *E. lucunter* can contribute greatly to the breakdown of coral reefs as a consequence of their burrowing behavior. *E. lucunter* is therefore considered a pest in many countries, including Brazil [16].

In this work, we report the purification, amino acid sequencing and structure prediction of a new RBL from *E. lucunter* eggs, named ELEL (*E. lucunter* eggs lectin). Based on structural data, the interaction of ELEL with carbohydrates was tested by molecular docking.

2. Materials and methods

2.1. Animal collection and extract preparation

Specimens of the rock boring sea urchin *E. lucunter* were collected at Pacheco Beach, Caucaia, Ceará State, Brazil. The animals were transported to the laboratory in a thermal box containing sea water.

E. lucunter eggs were obtained by coelomic injection of 0.5 M KCl. The eggs were dejelled by several washes in acidic seawater, pH 5.0. Dejelled eggs were defatted by treatment with cold acetone. After three exchanges of acetone, eggs become colorless.

Colorless eggs were suspended in five volumes of TBS (Tris-buffered saline, 150 mM NaCl, 0.1 mM PMSF, 50 mM Tris–HCl, pH 7.6) and sonicated in ice 10 times at 70 W for 50 s at intervals of 1 min using a Bandelin SONOPULS HD 2070 sonicator. The mixture was centrifuged at 5000 × *g* for 20 min at 4 °C. The supernatant was named crude extract and was assayed for hemagglutinating activity and protein concentration [17].

2.2. Purification of E. lucunter eggs lectin

The crude extract was loaded on a lactosyl-agarose column (1.0 cm × 3.0 cm), previously equilibrated with TBS. The column was washed with the same buffer at a flow rate of 1 mL/min until the column effluents showed absorbance of less than 0.01 at 280 nm. The adsorbed lectin was eluted with 0.3 M L-rhamnose in TBS. 1-mL fractions were manually collected, dialyzed and freeze-dried until use.

2.3. Hemagglutinating activity and hemagglutination inhibitory assay

Lectin activity was estimated by hemagglutinating activity against a solution of 3% rabbit and human erythrocytes (A, B and O) in native form and treated with proteases. The hemagglutination tests were performed in microtiter plates with V-bottom wells using the two-fold serial dilution method [18].

A hemagglutination inhibition assay was performed using the standard procedure [18]. The following carbohydrates and glycoproteins were used: D-fructose, D-galactose, D-glucose, D-mannose, L-rhamnose, methyl-α-D-galactopyranoside, methyl-α-D-galactopyranoside, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine, D-sucrose, D-melibiose, α-lactose, β-lactose, orosomucoid, ovomucoid, thyroglobulin and porcine stomach mucin (PSM). The initial concentrations of the inhibitors were 100 mM for sugars and 2 mg/mL for glycoproteins.

The effects of pH, temperature, EDTA and divalent cations on lectin activity were evaluated as described by Sampaio et al. [18].

2.4. Molecular mass and sugar content of ELEL

Molecular mass of ELEL under denaturing condition was estimated by SDS–PAGE in the presence and absence of β-mercaptoethanol, followed by staining with Coomassie Brilliant Blue, as described by Laemmli [19]. LMW-SDS Marker kit (GE Healthcare) was used as the standard (phosphorylase b (Mr: 97,000), albumin (Mr: 66,000), ovalbumin (Mr: 45,000), carbonic anhydrase (Mr: 30,000), trypsin inhibitor (Mr: 20,100) and α-lactalbumin (Mr: 14,400).

Glycoproteins in SDS–PAGE were stained with periodic acid-Schiff (PAS), as described by Zacharius et al. [20]. Neutral sugar content in ELEL was evaluated by phenol-sulfuric acid, using lactose as the standard [21].

The relative mass of native ELEL was estimated by gel filtration on a BioSuite 250 5 μm HR SEC (0.78 cm × 30 cm) column coupled to the H-Class Bio Acquity UPLC System (Waters Corp.). The column was equilibrated with 50 mM Tris–HCl, pH 7.2, containing 500 mM NaCl, and calibrated with conalbumin (75 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), ribonuclease A (13.7 kDa) and aprotinin (6.5 kDa).

The molecular mass of ELEL was determined by ElectroSpray Ionization-Mass Spectrometry (ESI-MS). Purified lectin (6 mg/mL) was solubilized in 8 M Urea and submitted to reverse phase chromatography (RPC) on a Sephasil Peptide C-8 10/250 column coupled to the H-Class Bio Acquity UPLC System (Waters Corp.). The column was equilibrated with 5% ACN in 0.1% trifluoracetic acid (TFA) and eluted with a gradient of acetonitrile (ACN) in 0.1% TFA. Fractions containing ELEL were collected and directly infused into a nanoelectrospray source coupled to a Synapt HDMS ESI-Q-ToF mass spectrometer (Waters Corp.). The instrument was calibrated with [Glu1]- Fibrinopeptide B fragments. Mass spectra were acquired by scanning at *m*/*z* ranging from 1000 to 4000 at 5 scans/s. The mass spectrometer was operated in positive mode, using a source temperature of 363 K and capillary voltage at 3.2 kV. Data collection and processing were controlled by Mass Lynx 4.1 software (Waters).

2.5. Primary structure determination by tandem mass spectrometry (MS/MS)

ELEL was submitted to SDS–PAGE as described above. After staining, ELEL spots were excised, reduced with dithiothreitol (DTT), and carboxyamidomethylated with iodoacetamide (IAA), as described by Shevchenko et al. [22].

Treated spots were subjected to digestion with the following enzymes: trypsin, chymotrypsin and pepsin. Digestions with trypsin and chymotrypsin were realized in 50 mM ammonium bicarbonate at 1:50 w/w (enzyme/substrate). Digestion with pepsin was performed in 0.1 M HCl at 1:50 w/w (enzyme/substrate). All digestions were maintained at 37 °C for 16 h.

The digestions were stopped with 2 μL of 2% formic acid (FA). The peptides were extracted from the gel conform, as described by Shevchenko et al. [22]. Two microliters of the peptide solution were loaded onto a C-18 (0.075 × 100 mm) nanocolumn coupled to a nanoAcquity system (Waters Corp.). The column was equilibrated with 0.1% FA and eluted with an ACN gradient in 0.1% FA. The eluates were directly infused into a nanoelectrospray source. The mass spectrometer was operated in positive mode with a source temperature of 373 K and a capillary voltage at 3.0 kV. LC–MS/MS was performed according to the data-dependent acquisition (DDA) method. The lock mass used in acquisition was *m*/*z* 785.84 ion of the [Glu1]- Fibrinopeptide B. The selected precursor ions were fragmented by collision-induced dissociation (CID) using argon as collision gas. All of the CID spectra were manually interpreted. A search for similar sequences was performed using the online tool