Plant Science 183 (2012) 20–26

Contents lists available at SciVerse ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Crataeva tapia bark lectin is an affinity adsorbent and insecticidal agent

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ARTICLE INFO

Article history:
Received 2 September 2011
Received in revised form 21 October 2011
Accepted 29 October 2011
Available online 4 November 2011

Keywords:
Crataeva tapia
Bark lectin
Affinity matrix
Termiticidal activity

ABSTRACT

Hemagglutinating activity has been associated to presence of lectin, carbohydrate-binding proteins. In this work *Crataeva tapia* bark lectin (CrataBL) was purified in milligram quantities (28 mg per g of bark) by ion exchange chromatography. The lectin was thermo-stable, ion-independent and N-terminal sequence analysis demonstrated similarity with miraculin and miraculin-like proteins (plant defensive proteins). Glycosylated nature of CrataBL was revealed using glycoprotein staining (periodic acid–Schiff's reagent), positive for polypeptides of apparent molecular masses 21 and 40 kDa on SDS-PAGE. Gel diffusion assay showed that glucose/mannose isolectins from *Cratylia mollis* recognized CrataBL glycan moiety. CrataBL hemagglutinating activity was inhibited by glycoproteins and CrataBL immobilized on cyanogen bromide-activated sepharose 4B (1 mL) bound 0.54 mg of glycoprotein (casein, fetuin and ovalbumin) per cycle. CrataBL was an insecticide agent against *Nasutitermes corniger* workers (termite that attack woods) with LC_{50} of 0.475 mg mL^{-1} for 6 days.

1. Introduction

Plant lectins comprise a structurally versatile group of carbohydrate binding proteins and this property may be used to isolate glycoproteins by affinity chromatography through immobilized lectins as adsorbent supports [1–4]. Lectin affinity adsorbents are able to fractionate both nitrogen and oxygen-linked sugar chain glycoproteins [2,5–7] and these matrices have been employed to separate cell surface glycoconjugates important for chemical diagnosis [8] and to separate glycoproteins by natural pattern of glycosylation [4].

Interaction of lectin with carbohydrates on different cellular surfaces can promote hemagglutination and other responses such as antimicrobial, mitogenic, anti-tumor and insecticidal activities [9,10]. The binding of lectin to glycosylated molecules present at digestive tract of insects reduces growth, delays the development and promotes mortality [11–13]. Lectins from *Myracrodruon urundeuva* bark, heartwood and leaf were termiticidal agents against *Nasutitermes corniger*, a soil-feeding and wood-damaging termite able to invade urban environment [14,15]. In Brazil, termites are responsible for approximately 42.7% of the overall damage to buildings. The economic losses exceed 200 and 800 million dollars a year in China and Japan, respectively, while in the United States these losses are above 1 billion a year [16,17]. Additional problem associated with termite damage is the cost of pesticides and their applications that have exceeded 1.5 billion dollars a year in United States [18]. Moreover, the use of synthetical pesticides has been associated with the emergence of resistant pest populations and damage to non-target organisms [19]. The investigation of termiticidal activity of natural and biodegradable compounds found in termite-resistant trees is a positive step in the search for new natural insecticidal agents.

Crataeva tapia (Capparidaceae) is a tree broadly distributed in Brazil occurring in Pluvial Tropical Atlantic Forest and Pantanal Tropical Forest. *C. tapia* is known by Brazilian people as "tapiá" or "cabaceira" and tapia fruit in English. Its wood is resistant to putrefaction and has been used in Brazilian civil construction and wooden canoe construction [20]. *C. tapia* bark has also used as food, fuel, forage, and medicine in Semi-Arid northeastern Region of Brazil [21].

Hemagglutinating activity from extract of *C. tapia* bark was previously isolated (yield of 80% for hemagglutinating activity and 56% for protein recovery) using a reversed micelle system of the anionic surfactant sodium di(2-ethylhexyl)sulfosuccinate in isooctane. The isolated protein was characterized as an oligomeric protein of 40 kDa and the reversed micelle system yielded lectin predominantly in the non-aggregated form of 21 kDa [22]. In this paper we describe the isolation of *C. tapia* bark lectin (CrataBL) by ion-exchange chromatography, procedure that produced CrataBL in

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doi:10.1016/j.plantsci.2011.10.018

milligram quantities predominantly in the native oligomeric form (40 kDa). Also, it is reported CrataBL N-terminal sequence, the use of lectin as affinity chromatography ligand and its insecticide activity against *N. corniger*.

2. Materials and methods

2.1. Plant material

C. tapia bark (Division Magnoliophyta, Class Magnoliopsida, Subclass Dilleniidae, Order Capparales, Family Capparaceae) was collected in Recife City, State of Pernambuco, northeastern Brazil. The bark was air dried, powdered (40 mesh) and stored at 4 °C.

2.2. Lectin isolation

Powdered bark (10 g) was suspended in 0.15 M NaCl (100 mL). A clear supernatant (crude extract) was obtained after homogenisation in a magnetic stirrer (16 h at 4 °C) followed by filtration through gauze and centrifugation (4000 × *g*, 15 min). The extract was evaluated for protein concentration and hemagglutinating activity. Soluble proteins in crude extract were fractionated with ammonium sulphate according to Green and Hughes [23]. The 30–60% precipitate fraction (30–60 F) was submitted to dialysis (3500 Da cut-off membrane, 4 °C) against distilled water (2 h) followed by 10 mM citrate–phosphate buffer pH 5.5 (2 h). The 30–60 F was loaded (11 mg of protein, hemagglutinating activity of 1024) onto a CM-cellulose (Sigma–Aldrich, USA) column (5.2 cm × 1.6 cm) equilibrated with 10 mM citrate–phosphate buffer pH 5.5 at flow rate of 20 mL h^{-1}. The unabsorbed proteins were eluted with equilibrating solution until the absorbance at 280 nm was lower than 0.05. Following the adsorbed hemagglutinating activity (CrataBL) was eluted with 0.5 M NaCl.

2.3. Protein content

Protein concentration was evaluated according to Lowry et al. [24] using bovine serum albumin (31.25–500 μg mL^{-1}) as standard.

2.4. Hemagglutinating activity and effect of inhibitors, temperature and ions on activity

Hemagglutinating assay was carried out in microtiter plates (Kartell S.P.A., Italy) according to Paiva and Coelho [25] using suspension (2.5%, v/v) of human (A, B and O types) or rabbit erythrocytes treated with glutaraldehyde [26]. One hemagglutination unit (titer^{-1}) was defined as the reciprocal of the highest dilution of sample promoting full erythrocyte agglutination. Specific hemagglutinating activity was defined as the ratio between the titer^{-1} and protein concentration (mg mL^{-1}).

Hemagglutinating activity of CrataBL was also evaluated after incubation (45 min) of lectin (50 μL) with different concentrations of monosaccharides (fructose, galactose, glucose, mannose, *N*-acetylgalactosamine and *N*-acetylglucosamine) or glycoproteins (casein, fetuin and ovalbumin) and at temperature of 30–100 °C. After incubation, the suspension of rabbit erythrocytes (50 μL) was added. Minimal inhibitory concentration was defined as the lowest concentration of monosaccharide or glycoprotein able to neutralize the hemagglutinating activity.

To evaluate the effect of divalent cations on CrataBL-induced hemagglutinating activity, the lectin was previously dialyzed against 5 mM EDTA (16 h at 4 °C) followed by 0.15 M NaCl (6 h at 4 °C) to eliminate EDTA. Subsequently, the hemagglutinating activity of dialyzed CrataBL was evaluated in the presence of 5 mM $CaCl_2$, $MnCl_2$ or $MgCl_2$ in 0.15 M NaCl.

2.5. Polyacrylamide gel electrophoresis (PAGE)

PAGE for native basic (7.5%, w/v, gel) and acidic (12%, w/v, gel) proteins was performed according to Reisfeld et al. [27] and Davis [28], respectively. Basic polypeptides were stained with 1% (w/v) Amido Black in 10% (v/v) acetic acid. Acidic polypeptides were stained with 0.02% (v/v) Coomassie Blue in 10% (v/v) acetic acid.

Electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) was performed on 10% (w/v) gels according to Laemmli [29]. After electrophoretic running, one gel was stained with Coomassie Brilliant Blue in 10% acetic acid (0.02%, v/v). The other gel was cut and polypeptides from CrataBL were submitted to glycoprotein staining with periodic acid–Schiff's reagent [30] and molecular mass markers (bovine serum albumin, 66,000 Da, ovalbumin, 45,000 Da, glyceraldehyde-3-phosphate dehydrogenase, 36,000 Da, carbonic anhydrase, 29,000 Da, trypsinogen, 24,000 Da, trypsin inhibitor, 20,000 Da, α-lactalbumin, 14,200 Da, aprotinin, 6500 Da from Sigma, USA) were stained with Coomassie Brilliant Blue in 10% acetic acid (0.02%, v/v).

2.6. Gel filtration chromatography

CrataBL was chromatographed by gel filtration on a Hiprep 16/60 Sephacryl S-300 column (16 mm × 60 cm)/Äkta FPLC system (Amersham Pharmacia Biotech, Sweden) pre-equilibrated at 24 °C with 0.5 M NaCl. Samples (2.0 mL containing 4 mg of protein) were injected and eluted (3.0 mL fraction) with 0.5 M NaCl at a flow rate of 0.5 mL min^{-1}. Bovine serum albumin (66,000 Da), ovalbumin (45,000 Da), trypsin inhibitor type III-O chicken (28,000 Da) and trypsin (25,000 Da) standards were similarly chromatographed.

2.7. N-terminal sequence

The N-terminal sequence analysis was performed by automated Edman degradation [31] on a Shimadzu PPSQ-23 Model Protein Sequencer. The homology of the sequences was searched using the BLAST protein sequence in NCBI database.

2.8. Gel diffusion

A gel diffusion assay was performed according to Ashford et al. [32]. The central well was charged (15 μg) with glucose/mannose-specific 1,4 isolectins from *Cratylia mollis* [33]. The sample wells were filled with crude extract (100 μg of protein), 30–60 F (100 μg of protein) or CrataBL (26 μg). The samples were allowed to diffuse in agarose gel [1% (w/v) in 0.15 M NaCl] in a humid chamber at 4 °C for 24 h after which the gel was exhaustively washed and stained with Coomassie Brilliant Blue [0.4% (w/v) in 25% (v/v) ethanol and 8% (v/v) acetic acid] for 15 min.

2.9. CrataBL immobilization on Sepharose 4B

Cyanogen bromide-activated Sepharose 4B was used for CrataBL immobilization according to Paiva et al. [1]. CNBr-activated Sepharose 4B (3 g) was washed with 0.5 M NaCl at pH 2.5, followed by 100 mM $NaHCO_3$ in 0.5 M NaCl, pH 8.2. The incubation (24 h at 4 °C) was performed with CrataBL (10 mg of protein). After filtration and washing with $NaHCO_3$ solution the CrataBL-Sepharose 4B matrix was washed with 100 mM $NaHCO_3$ in 0.5 M NaCl at pH 8.2 followed by 100 mM sodium acetate in 0.5 M NaCl at pH 4.0 and distilled water. CrataBL retention was determined by calculation of the difference between the weight of loaded CrataBL and the amount of protein found before filtration and washing.