International Biodeterioration & Biodegradation 65 (2011) 52–59

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Termiticidal activity of lectins from *Myracrodruon urundeuva* against *Nasutitermes corniger* and its mechanisms

Thiago H. Napoleão [a], Francis S. Gomes [a], Thâmarah A. Lima [a], Nataly D.L. Santos [a], Roberto A. Sá [c], Auristela C. Albuquerque [b], Luana C.B.B. Coelho [a], Patrícia M.G. Paiva [a,*]

[a] Laboratório de Glicoproteínas, Departamento de Bioquímica-CCB, Universidade Federal de Pernambuco, Cidade Universitária, 50670-420 Recife, Pernambuco, Brazil
[b] Departamento de Biologia, Universidade Federal Rural de Pernambuco, Dois Irmãos, 52171-030 Recife, Pernambuco, Brazil
[c] Centro Acadêmico do Agreste, Universidade Federal de Pernambuco, Nova Caruaru, 55002-970 Caruaru, Pernambuco, Brazil

ARTICLE INFO

Article history:
Received 24 March 2010
Received in revised form
6 May 2010
Accepted 7 May 2010
Available online 13 December 2010

Keywords:
Gut bacteria
Insecticidal activity
Lectin
Myracrodruon urundeuva
Nasutitermes corniger
Trypsin

ABSTRACT

The isolation of lectins from *Myracrodruon urundeuva* bark (MuBL) and heartwood (MuHL) as well as the termiticidal activity of MuHL against *Nasutitermes corniger* has already been described. This work reports on the purification of a leaf lectin (MuLL) and the characterization of MuBL, MuHL, and MuLL; also described are the resistance of hemagglutinating activity of the three lectins to trypsin activity from *N. corniger* gut and the termiticidal activity on *N. corniger* of MuBL (LC_{50} of 0.974 mg ml^{-1} on workers and 0.787 mg ml^{-1} on soldiers) and MuLL (LC_{50} of 0.374 mg ml^{-1} on workers and 0.432 mg ml^{-1} on soldiers). The antibacterial effect of MuBL, MuHL, and MuLL on bacteria from gut of *N. corniger* was also investigated and lectins showed similar bacteriostatic activity (MIC of 62.5 μg ml^{-1} for workers and 125 μg ml^{-1} for soldiers). MuBL and MuHL were more efficient bactericidal agents on bacteria in the workers' gut (MBC of 125 μg ml^{-1}) than MuLL (MBC of 250 μg ml^{-1}) and similar bactericidal activity was detected on bacteria in the gut of soldiers (MBC of 250 μg ml^{-1}). The termiticidal activity of *M. urundeuva* lectins can be explained by the chitin-binding property, resistance to termite digestive enzyme, and the antibacterial effect on symbiotic bacteria of *N. corniger* gut.

1. Introduction

Lectins are proteins able to agglutinate cells by recognition of glycoconjugates in cell surfaces (Correia et al., 2008). The ability of lectins to decipher the glycocode makes them unique cell-recognition markers for carbohydrates (Gemeiner et al., 2009). Lectins are widely distributed among plants, in which these proteins may play several physiological roles. Plant lectins have been isolated from bark, cladode, flower, heartwood, leaf, rhizome, root, and seed; advantages to the exploration of leaf as a lectin source are ease of tissue fragmentation that is performed before the protein extraction, and safety to the plant. Some plant lectins have an important role in activation of enzymes (Kestwal et al., 2007), in the nodulation mechanism of leguminous trees (Limpens and Bisseling, 2003), and in defence against microorganisms and insects (Peumans and Van Damme, 1995). Insecticidal lectins have been explored in biotechnology for insect control by genetic engineering aiming to increase resistance of plants (Couty et al., 2001; Sadeghi et al., 2008).

Plant lectins have been shown to interfere in feeding, development, reproduction, and survival of different life stages of insects (Macedo et al., 2002, 2007; Sauvion et al., 2004). Insecticidal activity of lectins has been demonstrated against many insects that cause losses in agriculture, industry, and other economic sectors (Macedo et al., 2007; Fitches et al., 2008; Sá et al., 2008) or are vectors of viruses that cause human diseases (Coelho et al., 2009; Sá et al., 2009b).

Several mechanisms of action for insecticidal lectins have been suggested. Lectins can interact with glycoconjugates on the membrane surface of epithelial cells along the digestive tract affecting signaling pathways, transport processes, and immune responses (Fitches et al., 2008). Eisemann et al. (1994) showed that the glucose-mannose lectin from *Canavalia ensiformis* (Con A) reduced the movement of nutrients and digestive enzymes across the peritrophic membrane of blowfly *Lucilia cuprina*. Chitin and glycosylated proteins containing *N*-acetylglucosamine residues of peritrophic membrane are targets for lectins with chitin-binding ability; these lectins interfere with the formation and integrity of the peritrophic membrane, affecting digestion and absorption of nutrients (Carlini and Grossi-de-Sá, 2002; Macedo et al., 2004, 2007). Other lectins cross the midgut epithelial barrier and pass into the insect circulatory system, resulting in a toxic action. Fitches et al. (2001)

* Corresponding author. Tel.: +55 8121268540; fax: +55 8121268576.
E-mail address: ppaivaufpe@yahoo.com.br (P.M.G. Paiva).

0964-8305/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibiod.2010.05.015

reported that Con A and the mannose-specific lectin from *Galanthus nivalis* promoted systemic effects via transport from gut contents to haemolymph of *Lacanobia oleracea* (tomato moth) larvae.

Termites have an important role in the cycling of carbon in the biosphere, but some species are responsible for deterioration of commercial wood, structures of buildings, paintings, ancient books, and monuments of historical importance. Korb (2007) estimated that the annual damage by termites reached U.S. $50 billion worldwide in 2005. Replacement of deteriorated wood increases tree cut and the impact of deforestation (Clausen and Yang, 2007). The soil-feeding and wood-damaging higher termite *Nasutitermes corniger* (Isoptera, Termitidae) is able to invade the urban environment, attacking wood in the structures of buildings (Paes et al., 2007).

Soil-feeding termites are able to digest the peptide component in soil humic substances by a combination of proteolytic activities and extreme alkalinity in their intestinal tract (Ji and Brune, 2005). Digestion of cellulose by termites is possible due to the presence in their gut of cellulases of endogenous origin (encoded by genes in the termite genome) or produced by hindgut symbionts (Breznak and Brune, 1994; Zhou et al., 2008). The main hindgut symbionts are cellulose-fermenting protozoa, except for higher termites (Termitidae family), in which the primary digestive role is displayed by prokaryotes (Tokuda et al., 1999, 2007). To control termites, plants have been screened as sources of compounds that affect the quantity of bacteria in termite gut (Ahmed et al., 2006).

Myracrodruon urundeuva is a tree broadly distributed in Brazil, popularly known as "aroeira do sertão" and "urundel"; it is one of numerous Caatinga plants with great importance in traditional medicine (Leite, 2002; Albuquerque and Oliveira, 2007). Analgesic, antibacterial, anti-inflammatory, and anti-ulcer activities have been reported for bark and heartwood constituents (Viana et al., 2003; Souza et al., 2006). Antioxidant, antifungal, and termite repellent activities have also been reported for secondary metabolites from heartwood (Sá et al., 2009a). Cationic lectins isolated from *M. urundeuva* bark (MuBL; 14.0 kDa) and heartwood (MuHL; 14.4 kDa) showed activity on fourth-stage larvae (L4) of *Aedes aegypti* and it was suggested that the larvicidal effect was due to interaction between the lectins and glycosylated components from insect peritrophic membrane (Sá et al., 2009b). Also, MuHL was able to induce mortality of *N. corniger* (LC_{50} values of 0.248 mg ml^{-1} for workers and 0.199 mg ml^{-1} for soldiers) and it was suggested that the lectin may play a role in the natural resistance of *M. urundeuva* wood, probably stored as a chemical protection (Sá et al., 2008). The resistance of hardwoods against insects and microorganisms has attracted researchers seeking to understand natural resistance and develop new natural insecticidal and antifungal compounds to be used as environmental friendly agents (Yen et al., 2008).

This work reports on the purification of *M. urundeuva* leaf lectin (MuLL); the isolation procedure of MuLL yielded highest amount of lectin per gram of tissue compared to those of bark and heartwood lectins. Structural and physicochemical characteristics of MuBL, MuHL, and MuLL are also shown. The resistance of lectin hemagglutinating activity to termite gut trypsin, the termiticidal activity on *N. corniger* of MuBL and MuLL, and the antimicrobial effect of *M. urundeuva* lectins on *N. corniger* gut bacteria are also covered.

2. Materials and methods

2.1. Plant material

Bark, heartwood, and leaves of *M. urundeuva* (Engl.) Fr. All. (Division Magnoliophyta, Class Magnoliopsida, Subclass Rosidae, Order Sapindales, Family Anacardiaceae) were collected in the state of Maranhão, in northeastern Brazil and a voucher specimen (identified by Mr. Gonçalo Mendes da Conceição) is deposited under number 054 at the Herbarium Aluisio Bittencourt, Centro de Estudos Superiores de Caxias, Universidade Estadual do Maranhão, Brazil. The tissues were air-dried, powdered (40 mesh), and stored at 28 °C.

2.2. Purification of MuBL, MuHL, and MuLL

The MuBL and MuHL were isolated according to Sá et al. (2009b). To purify MuLL, powdered leaves (10 g) were 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 (3000 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 (1955). The fractions were solubilised in 0.15 M NaCl and submitted to dialysis (3500 Da cut-off membrane, 4 °C) against distilled water (4 h) followed by 0.15 M NaCl (4 h). The 60–80% precipitate with highest hemagglutinating activity was loaded (2.0 ml; 47 mg of protein) onto a chitin (Sigma, USA) column (7.5 × 1.5 cm) equilibrated with 0.15 M NaCl (100 ml) at a flow rate of 20 ml h^{-1}. The unabsorbed proteins were eluted with equilibrating solution until the absorbance at 280 nm was negligible. Then the adsorbed hemagglutinating activity (MuLL) was eluted with 1.0 M acetic acid (140 ml), and exhaustively dialyzed against 0.15 M NaCl (1 l) for eluent elimination.

2.3. Hemagglutinating activity

Hemagglutinating assay was carried out in microtiter plates (Kartell S.P.A., Italy) according to Paiva and Coelho (1992) using a suspension (2.5% v/v) of human (A, B, AB, and O types) or rabbit erythrocytes treated with glutaraldehyde (Bing et al., 1967). One hemagglutination unit (titer) was defined as the reciprocal of the highest dilution of sample promoting full erythrocyte agglutination (Chumkhunthod et al., 2006). Specific hemagglutinating activity was defined as the ratio between the titer and protein concentration (mg ml^{-1}).

Hemagglutinating activity was also evaluated after incubation (30 min) of lectin (50 μl, 0.125 mg ml^{-1}) with 200 mM monosaccharide (arabinose, fructose, galactose, glucose, glucuronic acid, mannose, methyl-α-glucopyrannoside, methyl-α-mannopyranoside, *N*-acetylglucosamine, rhamnose, trehalose, and xylose) and 0.5 mg ml^{-1} glycoprotein (asialofetuin, azocasein, casein, fetuin, ovalbumin, and thyroglobulin), at a temperature of 30–100 °C and different pH values (citrate phosphate buffer, pH 4.0–6.0; sodium phosphate buffer, pH 7.0; Tris–HCl buffer, pH 8.0 and 9.0; NaOH solution, pH 10 and 11). After incubation the suspension of rabbit erythrocytes (50 μl) was added.

2.4. Chromatography of MuLL on N-acetylglucosamine column

To evaluate the interaction of MuLL with *N*-acetylglucosamine, the 60–80% precipitate (2.0 ml, 47 mg of protein) was loaded onto a column (7.5 cm × 1.5 cm) of N-acetyl-D-glucosamine immobilized in agarose gel (Sigma, USA) equilibrated at a 20 ml h^{-1} flow rate with 0.15 M NaCl (100 ml). A sample was applied and matrix was washed with equilibrating solution until the absorbance at 280 nm was negligible. The elution step was performed using 0.1 M sodium acetate in 0.15 M NaCl (30 ml), followed by 0.1 M glycine-HCl pH 2.6 (30 ml) and 0.1 M glycine-NaOH pH 9.0 (60 ml).

2.5. Polyacrylamide gel electrophoresis (PAGE) of MuLL

Polyacrylamide gel electrophoresis for native (10% w/v, gel) basic and acidic proteins was performed according to Reisfeld et al. (1962) and Davis (1964), respectively. Basic polypeptides were stained with