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Identification of Lectin Activity in the Hemolymph of *Castnia licus* Drury, a Sugar-Cane Giant Borer (*Lepidoptera-Castniidae*)

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ABSTRACT

Castnia licus Drury, in the larvae stage, is a major pest in the sugar-cane industry of Northeastern Brazil (giant borer). A natural hemagglutination activity was detected in the larvae hemolymph of this insect and the activity have not been inhibited by tested carbohydrates. Fractionation with ammonium sulfate showed that the supernatant of the 15–30% fraction (S15–30%) had the lectin's highest specific activity. Acidic and basic lectin activities were separated by chromatography on CM-cellulose; the fractionated molecular forms showed distinct electrophoresis mobilities.

Index Entries: Lectin; *Castnia licus*.

INTRODUCTION

Lectins are proteins of nonimmune origin that agglutinate cells through more than one carbohydrate binding site. Hydrophobic sites of noncarbohydrate binding nature also occur on these molecules (*1,2*). The biological

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material initially utilized for lectin purification was of plant origin, being distinguished from the seeds (*3*). Lectins have also been purified from microorganisms (*4*), vertebrates (*5,6*), and invertebrates (*7*). In the case of insects, pure lectins were mainly obtained from the hemolymph (*8–10*). *C. licus* is a holometabolous insect, and its biological cycle is completed in nearly 177 d; the giant borer larval stage correspond to 110 d (*11*). This report gives a first mention of natural hemagglutinating activity detected in the larvae hemolymph of this sugar-cane giant borer. Data indicate the presence of acidic and basic lectin molecular forms.

MATERIALS AND METHODS

The larvae were collected in the sugar mill Nossa Senhora das Maravilhas, Goiana, Pernambuco, Brazil; the hemolymph was obtained after cutting the larvae second proleg. Phenylthiourea was added to the hemolymph (0.5 mg/mL), which was then centrifuged for 10 min at 10,000*g*. The clean supernatant was frozen (prep 1). To microtiter plates, 50 μL of 150 m*M* NaCl were placed in each well, and 50 μL of samples were added, to obtain serial dilutions. Then 50 μL of cell suspension in 150 m*M* NaCl were added to each well. After resuspension, the material was left overnight at room temperature and read for lectin activity. The activities were determined using rabbit erytrocytes treated with glutaraldehyde (*12*). The activity was not inhibited by D(+)glucose, D(+)galactose, D(+)fucose, D(+)mannose, D(+)frutose, L(−)rhamnose, L(−)fucose, sucrose, lactose, *N*-acetyl α-D(−)glucosamine, α-methyl-D(−)mannoside, methyl-α-D(+) galactopyranoside and trehalose. Prep 1 was dialyzed against 10 m*M* citrate/phosphate buffer, pH 3.0. Ammonium sulfate was added for saturation at 0–10%, 10–15%, and 15–30%. The supernatant of the 15–30% fraction (S15–30%), after dialysis against the above-mentioned buffer, Prep 2, was passed through a CM-Cellulose column equilibrated with the buffer. The column was washed with the same buffer until A280 nm was 0.03. Elution was performed with a linear gradient of 0–0.4*M* NaCl in buffer (10 times the gel volume). Lectin preparations were submitted to electrophoresis on polyacrylamide gel against basic (*13*) and acidic (*14*) native proteins.

RESULTS

At pH 3.0 the highest titer (1024) and specific lectin activity (20.0) were obtained with Prep 1, and this activity was stabilized for at least 3 mo. The specific lectin activity is defined by the titer (the inverse of highest dilution showing total agglutination) divided by mg of protein/mL^{-1}. Prep 2 showed the highest lectin's specific activity (33.0) which was chromatographed on CM-Cellulose (Fig. 1). Peaks I (unadsorbed), II, and III

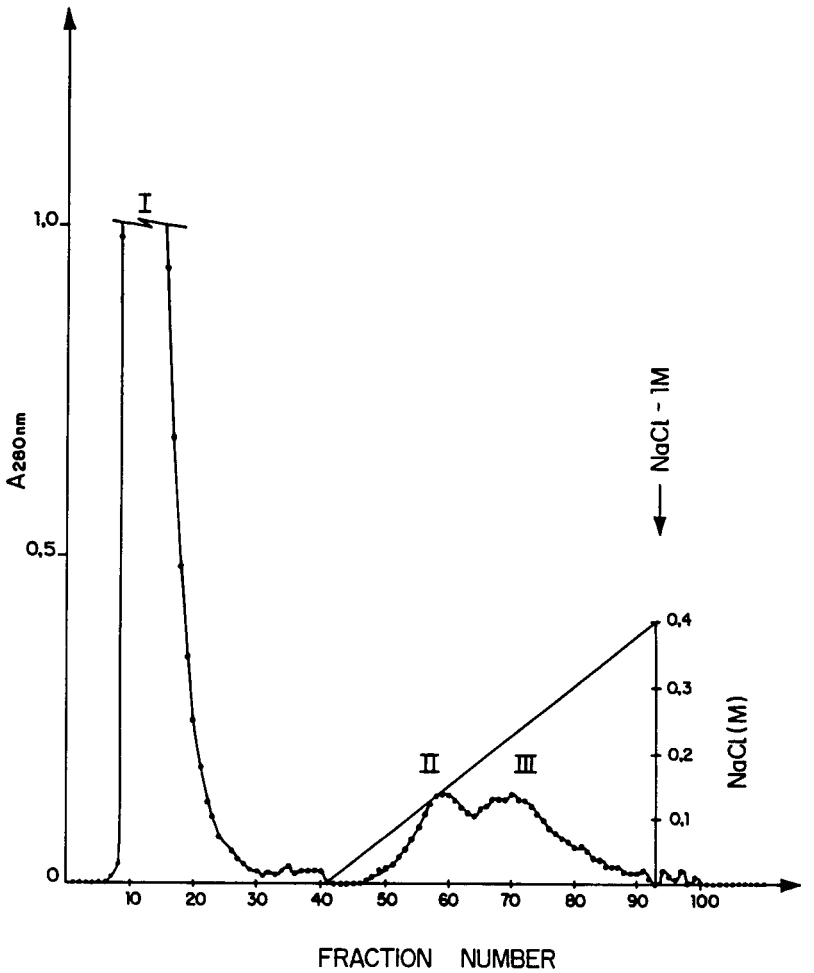

Fig. 1. Ion exchange chromatography of Prep 2. Sample: 20 mg of protein. Matrix: CM-Cellulose (9.6 mL). Flowrate: 10 mL/h. Collected fraction: 1.5 mL. Buffer: 10 m*M* citrate-phosphate, pH 3.0.

(adsorbed) were distinguished with lectin activity, but the highest activity was obtained in peak I. No relevant absorbance was registered when the column was washed with 1*M* NaCl. Polyacrylamide gel electrophoresis for basic proteins showed bands in peaks II and III (Fig. 2). The latter material seems very promising for the purification of a lectin in basic form from this insect. Peak I contained acidic proteins (not shown).

DISCUSSION

In hemolymph of ten lepidopteran larvae, moths, lectins were detected (*15*). It was speculated that lectins are present in all holometabolous insects (*16*). We worked in this study, with the giant borer *C. licus*, which is a

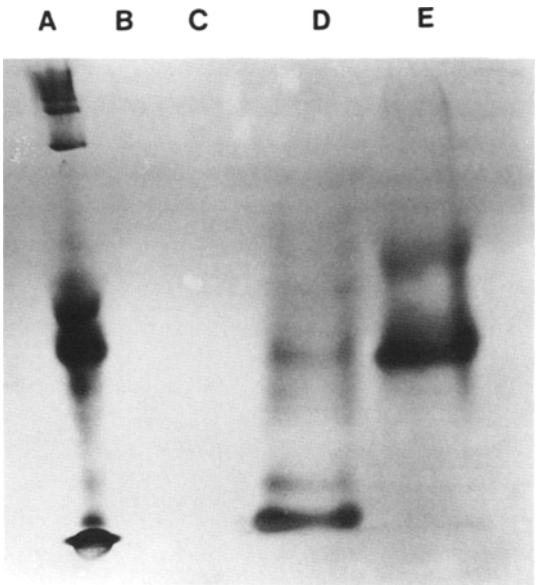

Fig. 2. Electrophoresis on polyacrylamide gel (7.5%) for basic protein. Prep 2, 400 μg of protein (**A**); peak I, fractions 9–16, 200 μg of protein (**B**) and fractions 17–24, 200 μg of protein (**C**); peak II, 264 μg of protein (**D**); peak III, 270 μg of protein (**E**). Staining: 1% (w/v) Amido Black in 10% (v/v) acetic acid.

holometabolous moth. The purification of lectins or multiple molecular forms of lectins has included initial conventional purification approaches such as ammonium sulfate fractionation and ion exchange chromatography. The mentioned forms, of distinct genetic origin, isolectins (*8,17–19*), or nondefined genetic origin, isoforms (*20*), have been isolated from different natural sources. The present communication contributes to register heterogeneity in insect lectins.

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