JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Purification of a Lectin from the Marine Red Alga *Gracilaria cornea* and Its Effects on the Cattle Tick *Boophilus microplus* (Acari: Ixodidae)

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A lectin was purified from the seaweed *Gracilaria cornea* by hydrophobic interaction chromatography on phenyl-Sepharose CL-4B followed by affinity chromatography on immobilized mucin. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis of *G. cornea* lectin (GCL) revealed a single protein band of ∼60 kDa, whereas by gel filtration on Sephadex G-100 its native molecular mass was 66 kDa. GCL exhibited a single isoeletric point of 4.3 and a 52.5% content of neutral sugars. Furthermore, the EDTA-treated lectin did not show any significant decrease in its ability to agglutinate trypsin-treated chicken erythrocytes. These data suggest that GCL is an acidic, monomeric glycoprotein that probably does not require divalent metal ions for its hemagglutinating activity. GCL hemagglutinating activity was not inhibited by any of the mono-, di-, and trisaccharides tested but was by the complex glycoproteins fetuin and porcine stomach mucin. Exposure of engorged females of the cattle tick (*Boophilus microplus*) to 0.1 mg mL^{-1} GCL significantly ($P < 0.05$) reduced the female weight after the oviposition period, the egg mass weight, the hatching period, and the mean larvae survival time.

KEYWORDS: ***Gracilaria cornea*; lectin; characterization; *Boophilus microplus*; acaricidal activity**

INTRODUCTION

Lectins are proteins or glycoproteins that possess at least one noncatalytic domain that interacts reversibly to a specific mono- or oligosaccharide (*1*, *2*). As a consequence of their properties, they have become useful tools in several fields of biological research such as immunology, cell biology, membrane structure studies, cancer research, and genetic engineering. They have been studied in almost all living organisms, especially in land plants (*2*). Boyd et al. (*3*) were the first to report the occurrence of lectins in marine algae. Since then, several studies on the purification of lectins from seaweeds have been reported, but the number of these proteins purified and characterized is still small in comparison to lectins from higher plants, for example (*4*).

Algal lectins differ from higher plant lectins in a variety of physicochemical characteristics. In general, algal lectins are monomeric, low molecular weight proteins, exhibiting a high content of acidic amino acids, with isoeletric points in the range of 4−6, and not requiring divalent metal cations for their biological activities. Furthermore, their carbohydrate-binding specificity is usually complex, meaning that they do not bind to simple sugars but have high affinities for complex oligosaccharides, especially those found in animal glycoproteins (*4*−*6*). Compared to plant lectins, there are only a few reports on the use of marine algal lectins. For example, some seaweed lectins have been shown to strongly agglutinate mouse FM3A tumor cells in lower concentrations than those required for lectins from land plants (*7*, *8*). Dalton et al. (*9*) found that the prepurified lectins of some marine algae exhibited high mitogenic activity for human lymphocytes. Furthermore, Griffin et al. (*10*) demonstrated the use of *Codium fragile* lectin conjugated to collodial gold as a new histochemical reagent.

Ticks are blood feeder arthropods, distributed into two major families, the Argasidae and the Ixodidae (*11*). Many tick species from the Ixodidae family are important vectors of human and animal diseases such as Mediterranean spotted fever transmitted by *Rhipicephalus sanguineus* (*12*), Lyme disease transmitted by *Ixodes dammini* (*13*, *14*), monocytic and granulocytic ehrlichiosis transmitted by *Ixodes ricinus* (*15*), and the cattle

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10.1021/jf0509660 CCC: $30.25
Published on Web 07/06/2005

diseases anaplasmosis and babesiosis, which are both transmited by *Boophilus microplus* (*16*).

Ticks differ from other hematophagous animals in the period they remain attached to their vertebrate hosts, which ranges from several days to weeks. This long period obligates the tick to produce many different substances to interfere in the host defenses, including inflammatory and immune responses (*17*, *18*). The ectoparasite *B. microplus* is a major veterinary problem as a vector of severe diseases and as a debilitating agent to cattle in tropical and subtropical areas of the world (*19*). The traditional control method for *B. microplus* includes the use of acaricides, and a variety of chemicals have been used for this purpose. However, the selection for resistant tick strains and the potential environmental hazard that follows the use of these conventional acaricides show the need for the evaluation of new compounds.

In the present work we describe the purification and partial characterization of a new lectin from the marine red alga *Gracilaria cornea* and its effects on the cattle tick (*B. microplus*).

MATERIALS AND METHODS

Algal Material. The red alga *G. cornea* J. Agardh (Gracilariaceae, Rodophyta) was collected from the Atlantic coast at Mucuripe Beach in the city of Fortaleza, State of Ceará, Brazil. Voucher specimens are deposited in the Herbarium Prisco Bezerra, Universidade Federal do Ceará (UFC), Fortaleza, Ceará. After collection, the material was cleaned to remove epiphytes, washed with distilled water, and stored at −20 °C until used.

Blood Cells. Human blood samples (ABO system) were obtained from healthy donors at the Hematology Center of the UFC. Rabbit red blood cells were obtained by venous puncture from healthy animals reared at the Departamento de Bioquímica e Biologia Molecular, UFC. Chicken blood was obtained from healthy animals reared at the Departamento de Zootecnia, UFC.

Cattle Ticks. Cattle ticks (*B. microplus*) were obtained from infested animals reared at the Faculdade de Veterinária (FAVET), Universidade Estadual do Ceará (UECE), Fortaleza, Ceará, Brazil. The assays were performed on fully engorged female ticks obtained from infested cattle, which had been mantained for 60 days without application of acaricides.

Lectin Purification. To purify the *G. cornea* lectin (GCL), algal material was ground to a fine powder in a mortar and pestle in the presence of liquid nitrogen. The powder was then stirred (1:3 w/v) with 0.025 M Tris-HCl buffer, pH 7.5 (TB), for 18 h at room temperature. Insoluble algal material was removed by filtration using a nylon membrane followed by centrifugation at 10000*g* for 30 min at 4 °C. The crude, clear extract was partially concentrated by lyophilization and submitted to hydrophobic interaction chromatography (HIC) on a column of phenyl-Sepharose CL-4B (15 × 1.2 cm), equilibrated and eluted with TB containing 4 M NaCl at a flow rate of 30 mL h^{-1}. After elution of unbound proteins in the equilibrium buffer, adsorbed proteins were eluted with a decreasing gradient of NaCl (from 4 to 0 M) in TB, after which the matrix was washed with distilled water. The active fractions (containing hemagglutinanting activity) were pooled, dialyzed against TB, and submitted to affinity chromatography on a mucin−Sepharose 4B column. The gel (5.0 × 1.5 cm) was equilibrated and eluted with TB, followed by elution of the adsorbed lectin by the addition of 4 M urea.

Fractions with hemagglutinating activity were pooled, dialyzed against distilled water, freeze-dried, and stored at room temperature until used.

Protein Concentration. The content of soluble proteins was determined according to the Bradford assay (*20*) using bovine serum albumin (BSA) as a standard. Absorbance at 280 nm was used to estimate protein content in chromatographic fractions.

Carbohydrate Content. The amount of neutral sugars in the purified lectin was estimated according to the phenol−sulfuric acid method (*21*) using D-glucose as standard.

Hemagglutination and Hapten Inhibition Tests. Lectin-mediated agglutination of red blood cells from humans or animals was determined as described previously (*22*). Samples to be tested (100 μL) were assayed in small glass test tubes using 2-fold serial dilutions in 0.1 M Tris-HCl, pH 7.6, containing 0.15 M NaCl. A 2% suspension of trypsin-treated erythrocytes was added to each tube for a final volume of 200 μL. The mixture was then incubated at 37 °C for 30 min followed by another 30 min interval at room temperature. The hemagglutination titer (HU mL^{-1}) was recorded as the reciprocal of the highest dilution still giving visible agglutination. Specific activity was expressed as hemagglutination units (HU) per milligram of soluble protein [HU (mg of protein)−1]. The carbohydrate-binding specificity of the purified lectin was assessed by the ability of a series of sugars (L- and D-arabinose, D-cellobiose, D-fructose, L-fucose, D-galactose, D-glucose, D-raffinose, L-rhamnose, D-mannose, D-mannosamine, α-D-melibiose, D-melezitose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, α-D-glucosamine, D-glucuronic acid, D-ribose, sucrose, D-trehalose, and D-xylose) and glycoproteins (avidin, fetuin, ovalbumin, and porcine stomach mucin) to inhibit its hemagglutination activity against trypsin-treated erythrocytes from chicken. All sugars and glycoproteins were purchased from Sigma. The hapten inhibition tests were carried out by 2-fold serial dilutions of sugars (0.1 M) or glycoprotein (5 mg mL^{-1}) solutions in 0.15 M NaCl with a final volume of 100 μL. An equal volume of lectin solution containing 4 HU was added to each tube and the mixture allowed to interact for 1 h at room temperature. A 2% suspension of trypsin-treated erythrocytes from chicken was added to each tube for a final volume of 200 μL, and this mixture was incubated at 37 °C for 30 min followed by another 30 min interval at room temperature. The lowest concentration of a specific sugar or glycoprotein that inhibited hemagglutination (minimum inhibitory concentration, MIC) was recorded and used to define inhibitory potency.

Effects of EDTA and Divalent Cations (Ca^{2+} and Mn^{2+}) on Lectin Hemagglutinating Activity. The purified, active lectin was dialyzed against 5 mM EDTA in 0.15 M NaCl for 16 h at 8 °C and then submitted to hemagglutination activity tests as previously described. The hemagglutination assays were carried out either in the presence or in the absence of the divalent cations Ca^{2+} and Mn^{2+}. This was achieved by submitting the EDTA-treated lectin to 2-fold serial dilutions in 0.15 M NaCl with or without 5 mM $CaCl_2$ and 5 mM $MnCl_2$. The hemagglutinating activity was measured by the addition of trypsin-treated chicken erythrocytes.

Heat Stability of Lectin. The heat stability of GCL was determined by incubating aliquots from a lectin solution (2 mg mL^{-1}) at 40, 50, and 60 °C for 2.5, 5, 10, 20, 30, 40, and 60 min. The samples were then cooled and assayed for hemagglutinating activity as described before. The free energy change ($\Delta G'$) of activation of the lectin denaturation process was determined using the expression of Arrhenius (*23*). The velocity constant of the reaction (k_1) was first determined as the slope of the curve obtained by the expression $k_1 t = -\ln A/A_0$, where A = residual hemagglutinanting activity after heat treatment, A_0 = initial hemagglutinating activity before heat treatment, and t = time of heat treatment (in seconds). The velocity constant k_1 is related to the standard free energy change by the following formula: $\Delta G' = RT \ln(kT/k_1 h)$, where R is the gas constant (1.987 cal mol^{-1} K^{-1}), T is the absolute temperature (K), k is the Boltzmann constant (1.37×10^{-6} erg K^{-1}), k_1 is the velocity constant, and h is Planck's constant (6.25×10^{-27} erg s^{-1}).

Molecular Weight Determination. The native molecular mass of GCL was determined by gel filtration chromatography on a Sephadex G-100 column (1.6 × 89 cm) equilibrated and eluted with TB. Bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa), and cytochrome *c* (12.4 kDa) were used as standard proteins. The void volume (V_0) was estimated with Blue Dextran (Sigma Chemical Co.). Molecular mass estimation under denaturing conditions was carried out by discontinuous electrophoresis using a vertical system following the Laemmli method (SDS-PAGE) as described by Hames and Rickwood (*24*). A 12.5% polyacrylamide slab in 0.025 M Tris-HCl, 0.2 M glycine, pH 8.9, with 0.1% (w/v) sodium dodecyl sulfate was used. Samples and standards were prepared in Tris-HCl buffer, pH 6.8, containing 2% (w/v) SDS and 5% (v/v) 2-mercaptoethanol, followed by heating at 100 °C for 10 min. BSA (66 kDa), ovalbumin (45 kDa), glycer-

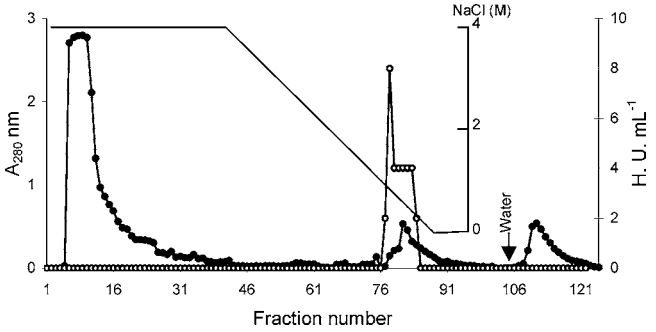

Figure 1. Hydrophobic interaction chromatography of the crude extract of the marine red alga *G. cornea* on a phenyl-Sepharose CL-4B column. The sodium chloride concentration in the crude extract was made up to 4 M, and the solution was applied to the phenyl-Sepharose CL-4B column, equilibrated with 25 mM Tris-HCl buffer, pH 7.5, containing 4 M NaCl. The first peak (PI) was eluted with the equilibrium buffer, the second peak (PII) was eluted with a decreasing gradient of NaCl (from 4 to 0 M), and the third peak (PIII) was eluted with distilled water: (●) absorbance at 280 nm; (○) hemmaglutinating activity, HU mL^{-1}. The hemagglutinating activity is expressed as the reciprocal of the highest dilution still giving visible agglutination of trypsin-treated chicken erythrocytes.

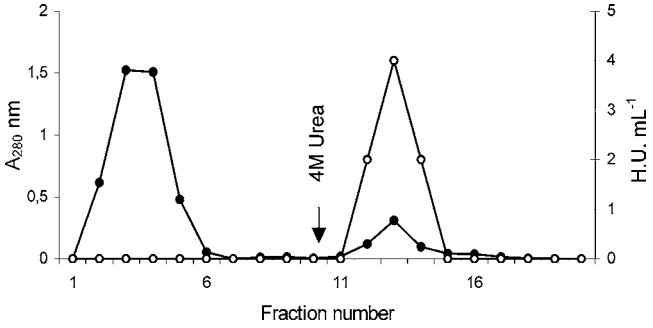

Figure 2. Affinity chromatography of *G. cornea* lectin on a mucin–Sepharose 4B column: (●) absorbance at 280 nm; (○) hemagglutinating activity, HU mL^{-1}. Peak II from the hydrophobic interaction chromatography was applied to the column equilibrated and eluted with 0.025 M Tris-HCl buffer, pH 7.5, to remove unbound proteins. The adsorbed lectin was eluted with 4 M urea. The hemagglutinating activity is expressed as the reciprocal of the highest dilution still giving visible agglutination of trypsin-treated chicken erythrocytes.

aldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactoalbumin (14.4 kDa) were used as standard proteins. A standard Coomassie blue method was used for staining protein bands following electrophoresis.

Isoelectric Point Determination. Isoelectric focusing (IEF) experiments (*25*) were carried out using Immobiline DryStrip gels pH 3−10, 11 cm long, and Pharmalyt (IPG buffer) pH 3−10 buffer (Amersham Biosciences Corp.) according to instructions provided by the manufacturer. After the run, the gel was equilibrated for 15 min in 50 mM Tris buffer, pH 8.8, 30% (w/v) glycerol, 6 M urea, 2% (w/v) SDS, 65 mM DTT, and 0.001% (w/v) bromophenol blue and used immediately for SDS-PAGE (second dimension).

Acaricidal Assays. The acaricidal effects of the crude extract of *G. cornea*, peak II obtained when the crude extract was submitted to hydrophobic interaction chromatography (PII-HIC), and the purified *G. cornea* lectin were investigated. Five engorged female ticks (three replicates for each treatment as well as for the control) were weighed collectively and immersed for 5 min in *G. cornea* crude extract (containing 2.6 mg of protein mL^{-1}), PII-HIC (containing 0.59 mg of protein mL^{-1}), or a solution of pure lectin (in a concentration of 0.1 mg mL^{-1}). Control ticks were immersed in distilled water. The treated and control ticks were kept in Petri dishes (9 cm diameter) in an incubator at 27 ± 2 °C and 80% relative humidity. Females were allowed to oviposit, and the eggs obtained from each female on each day were weighed collectively, transferred to a vial (2 × 10 cm) covered with a moist cotton plug, and incubated in the same conditions as described before. After the oviposition period had finished, females were weighed again and the percentage of eggs hatched and larval mortality were determined by visually estimating the proportion of larvae in relation to the proportion of unhatched eggs in the vial. All data were submitted to one-way analysis of variance (ANOVA), and the means were compared by Tukey's test.

RESULTS AND DISCUSSION

Crude extracts of the red alga *G. cornea* agglutinated trypsin-treated red blood cells from chicken (64 HU mL^{-1}), but no agglutination was observed against rabbit and human erythrocytes (blood groups A, B, and O), even when the cells were treated with trypsin. GCL was then purified by a combination of hydrophobic interaction and affinity chromatographies. When the concentrated, buffered crude extract was submitted to hydrophobic interaction chromatography on a phenyl-Sepharose CL-4B column (**Figure 1**), a retained peak (PII) containing all hemagglutinating activity was bound to the matrix and eluted with a decreasing gradient of sodium chloride in the equilibrium buffer. In addition to the 4.4-fold purification achieved (**Table 1**), this chromatographic step was very effective in eliminating red pigments, because most of them did not bind to the Sepharose matrix. Further purification of the lectin was achieved by affinity chromatography on mucin−Sepharose 4B (**Figure 2**). As result, a 10.5-fold increase in the specific activity (**Table 1**) was obtained. Actually, affinity chromatography on immobilized mucin has been shown to be very effective in the purification of algae lectins (*22*, *26*, *27*).

The purified GCL showed a single protein band with an apparent molecular mass of ∼57 kDa when submitted to SDS-PAGE under reducing and nonreducing conditions (**Figure 3**). The native molecular mass of GCL as determined by gel filtration on a Sephadex G-100 column was 66 kDa, thus suggesting that the lectin is a monomeric protein. Most marine algal lectins are low molecular weight proteins (*4*), and values as low as 3.5 and 4.5 kDa were reported for the lectins from the red algae *Bryothamnion triquetrum* and *Bryothamnion seaforthii*, respectively (*28*). Algal lectins with molecular masses closer to that of GCL include those from *Ptilota serrata* (64.5 kDa; *29*) and *Palmaria palmata* (62.0 kDa; *30*).

Table 1. Purification of the Lectin from the Marine Red Alga *G. cornea*

fraction	total protein (mg)	total activity[a] (HU mL^{-1})	specific activity[b] [HU (mg of protein)$^{-1}$]	yield (%)	MAC[c] (μg mL^{-1})	purification (fold)
crude extract	139.2	5632	49.0	100.0	20.0	1.0
PII-HIC	7.1	1536	216.3	27.3	4.6	4.4
PII of mucin–Sepharose	0.25	256	516.1	4.5	3.9	10.5

[a] Inverse of the highest dilution still causing agglutination of trypsin-treated chicken erythrocytes. [b] Hemagglutination units per milligram of protein. [c] Minimum agglutination capacity (minimum amount of protein that is able to agglutinate trypsin-treated chicken erythrocytes).

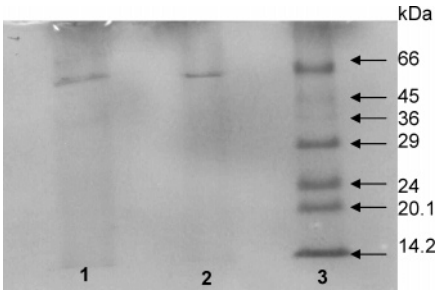

Figure 3. SDS-PAGE of the purified lectin from *G. cornea*: (lane 1) purified lectin treated with 2-mercaptoethanol; (lane 2) purified lectin not reduced with 2-mercaptoethanol; (lane 3) standard proteins (α-lactoalbumin, 14.4 kDa; soybean trypsin inhibitor, 20.1 kDa; trypsinogen, 24.0 kDa; carbonic anhydrase, 29.0 kDa; glyceraldehyde-3-phosphate dehydrogenase, 36.0 kDa; ovalbumin, 45.0 kDa; bovine serum albumin, 66.0 kDa).

Table 2. Hapten Inhibition of the Hemagglutinating Activity of *G. cornea* Lectin

glycoproteins and simple sugars	minimum inhibitory concentration[a] (μg mL^{-1})
fetuin	3.12
porcine stomach mucin	6.25
avidin	NI
ovalbumin	NI
simple sugars[b]	NI
disaccharides[c]	NI
trisaccharides[d]	NI

[a] Lowest concentration of a specific sugar/glycoprotein that inhibited one hemagglutinating unit (HU) of purified *G. cornea* lectin when assayed with trypsin-treated chicken erythrocytes. NI, no inhibition at 25 mM. [b] D-Glucosamine, D-mannose, D-ribose, L- and D-arabinose, L-rhamnose, D-fructose, D-galactose, D-glucuronic acid, D-mannosamine, L-fucose, methyl α-D-mannopyranoside, D-glucose, methyl α-D-glucopyranoside, and D-xylose. [c] D-Cellobiose, α-D-melibiose, trehalose, and sucrose. [d] D-Raffinose and D-melezitose.

The GCL was found to be an acidic protein with an estimated p*I* of 4.3, and the carbohydrate analysis according to the phenol−sulfuric acid method showed that the lectin contains 52.5% of neutral sugars. Furthermore, divalent cations seem to be not required for GCL hemagglutinating activity as the hemagglutination titer of EDTA-treated lectin did not show any significant difference in relation to the native protein. Purified GCL was stable at 40 °C for 20 min and retained 12.5% of its original activity after 60 min at this temperature. However, its hemagglutinating activity was totally abolished after heat treatment at 50 °C for 5 min or at 60 °C for 2.5 min (**Figure 4**), showing that GCL is very sensitive to temperature in comparison to most lectins characterized. The free energy change ($\Delta G'$) of activation of the denaturation process was

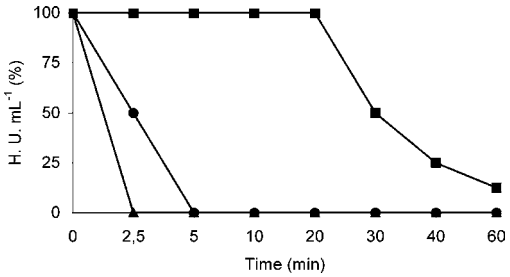

Figure 4. Effect of temperature on the hemagglutinating activity of *G. cornea* lectin: (■) 40 °C; (●) 50 °C; (▲) 60 °C. The residual lectin activity along heat treatments is shown as the percentage of hemagglutinating units (HU) at each time in relation to the hemagglutinating titer (HU mL^{-1}) of the native protein.

estimated to be 97.0 kJ mol^{-1}. In contrast, the seaweed lectin from *Vidalia obtusiloba*, which is completely inactivated when heated at 90 °C for 30 min, has a $\Delta G'$ of activation of the denaturation process of 106.3 kJ mol^{-1} (*31*). Because many lectins exert antinutritional effects on mammals including humans (*32*), the determination of the free energy of activation of the lectin denaturation process is an important physicochemical parameter considering the increasing applications of lectins in agriculture, medicine, and related areas. This information is also relevant from the nutritional point of view, taking into account the potential use of seaweeds in human and animal nutrition.

GCL hemagglutinating activity was not inhibited by any of the mono-, di-, and trisaccharides tested (**Table 2**), but only by the glycoproteins fetuin (MIC = 3.12 μg mL^{-1}) and porcine stomach mucin (MIC = 6.25 μg mL^{-1}). Avidin and ovalbumin did not show any inhibitory activity on GCL hemagglutinating activity. Porcine stomach mucin is an O-linked glycoprotein with glycans rich in nonreducing terminal galactose residues, as well as fucose and galactose as internal residues, whereas fetuin is an N-linked glycoprotein of the complex type with units of the disaccharide Galβ4GlcNAc (*N*-acetyllactosamine) substituted with sialic acid, attached to the pentasaccharide core (*33*). Therefore, the carbohydrate-binding specificity of GCL is complex, and its inhibition by porcine stomach mucin is a feature that has also been reported for other algal lectins such as those from *Enantiocladia duperreyi*, *Caulerpa cupressoides*, *Ptilota filicina*, and *Pterocladiella capillacea* (*22*, *27*, *34*, *35*).

The deleterious effects of many lectins, most of them from higher plants, on insect pests (Arthropoda, class Insecta) is now well established, as reviewed by Vasconcelos and Oliveira (*32*). Indeed, plant lectins are considered to be defense proteins against the herbivory of insects and mammals, for example (*1*, *2*, *32*). Although lectins have been found and studied in some tick species (*36*), no studies have yet been published on any possible

Table 3. Effects of Proteic Fractions and Purified Lectin from the Marine Red Alga *G. cornea* on Biological Parameters of the Cattle Tick (*B. microplus*)[a]

biological parameters	control	crude extract[b]	PII-HIC[c]	lectin (0.1 mg mL^{-1})
female weight after oviposition (mg)	59.4 ± 6.7a	56.4 ± 5.3a	59.8 ± 12.5a	47.5 ± 4.1b
egg mass weight (mg)	151.1 ± 7.7a	146.6 ± 12.3a	148.8 ± 12.2a	93.9 ± 12.8b
egg hatching (%)	96.3 ± 1.8a	88.0 ± 9.0b	94.1 ± 4.5a	96.8 ± 1.2a
oviposition period (days)	12.0 ± 1.3a	10.2 ± 1.2b	11.0 ± 1.2a	11.0 ± 1.0a
incubation of eggs (days)	20.0 ± 3.7a	22.5 ± 2.0a	22.0 ± 0.4a	24.0 ± 0.7a
egg hatching period (days)	22.0 ± 2.3a	16.3 ± 2.7b	13.6 ± 2.9c	9.4 ± 1.5d
mean larvae survival time (days)	98.0 ± 7.0a	72.7 ± 4.1b	77.3 ± 6.5c	67.3 ± 6.6d

[a] Numbers followed by different letters in each row differ significantly from each other by Tukey's test ($p < 0.05$). [b] Crude extract containing 2.6 mg of protein mL^{-1}. [c] Peak II (containing 0.59 mg of protein mL^{-1}) of the hydrophobic interaction chromatography of *G. cornea* extract.

acaricidal effects of exogenous lectins on ticks (Arthropoda, class Arachnida). To investigate this, proteic fractions (crude extract and PII-HIC) from *G. cornea* seaweed as well as the purified GCL were tested against the cattle tick (*B. microplus*). At 0.1 mg mL^{-1} GCL caused a 20% reduction in female weight afer the oviposition period, and this was significant ($P < 0.05$) in relation to control ticks (**Table 3**). GCL significantly ($P < 0.05$) affected (37.9% reduction) the mean egg mass weight laid by treated, engorged females in relation to the value observed in control ticks (**Table 3**). In addition, significant ($P < 0.05$) reductions in the hatching period and the mean larvae survival time (57.3 and 31.3%, respectively) were also observed by exposure of engorged females to GCL. No significant effects ($P > 0.05$) of GCL were observed on the percentage of egg hatching, oviposition period, and egg incubation period. These data show that *G. cornea* lectin has acaricidal activity, and this property should be further investigated as lectins may constitute a new natural source for the control of these economically important parasites.

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Received for review April 26, 2005. Revised manuscript received June 8, 2005. Accepted June 9, 2005. This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP).

JF0509660