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Structural analysis and anthelmintic activity of *Canavalia brasiliensis* lectin reveal molecular correlation between the carbohydrate recognition domain and glycans of *Haemonchus contortus*

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ABSTRACT

Haemonchus contortus is one of the most economically important parasites infecting small ruminants worldwide. This nematode has shown a great ability to develop resistance to anthelmintic drugs, calling for the development of alternative control approaches. Because lectins recognize and bind to specific carbohydrates and glycan structures present in parasites, they can be considered as an alternative to develop new antiparasitic drugs. Accordingly, this work aimed to investigate the anthelmintic effect of *Canavalia brasiliensis* (ConBr) lectin against *H. contortus* and to evaluate a possible interaction of ConBr with glycans of this parasite by molecular docking. ConBr showed significant inhibition of *H. contortus* larval development with an IC_{50} of 0.26 mg mL^{-1}. Molecular docking assays revealed that glycans containing the core trimannoside [Man(α1-3)Man(α1-6)Man] of *H. contortus* interact in the carbohydrate recognition domain of ConBr with an interaction value of MDS = −248.77. Our findings suggest that the inhibition of *H. contortus* larval development is directly related to the recognition of the core trimannoside present in the glycans of these parasites. This work is the first to report on the structure-function relationships of the anthelmintic activity of plant lectins.

1. Introduction

Haemonchus contortus, a common nematode infecting small ruminants, is considered one of the most economically important parasites worldwide [1,2]. In recent years, the resistance of these parasites to anthelmintic drugs has increased, owing to the continuous use of the same drug and use of anthelmintics in suboptimal doses [3].

Therefore, the anthelmintic resistance in *H. contortus* increases the need to develop new intervention strategies. Insights into the biological processes of *H. contortus* at the molecular level might identify key molecules as new drug targets, contributing to the development of alternative control drugs [4,5].

In this context, the glycans of parasites have attracted attention by their unusual structures and their potential roles in immunomodulation and protective immunity, as target molecules for antiglycan antibodies or lectins with antiparasitic effects [6].

Lectins are carbohydrate-binding glyco/proteins that are capable of agglutinating cells without enzymatic activity toward carbohydrate or glycoconjugates [7]. Thus, lectins play a role in immunology and glycobiology where they are used to agglutinate cells and identify complex carbohydrates and glycoconjugates, but lectins have also been shown to mediate diversified biological functions like cytotoxicity, complement activation, cell-to-cell and host-pathogen communications, innate immune responsiveness, and cell-to-cell signaling [8].

The binding of lectins to carbohydrates is noncovalent and reversible, involving hydrogen bonds, hydrophobic, electrostatic and van der Waals interactions and dipole attraction [9]. The binding of monosaccharides (*i.e.* mannose, glucose, and galactose) and

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disaccharides (sucrose and lactose) to a lectin is relatively weak, with dissociation constants in the millimolar or micromolar range. On the other hand, interactions of multivalent lectins with complex, branched carbohydrates (*i.e.* fetuin, asialofetuin, mucin and high-mannose glycans) containing multiple epitopes result in high-avidity binding with nanomolar or even picomolar dissociation constants [10]. This makes these proteins ideal molecular tools for the study of protein-glycan interaction of various parasites [11].

ConBr is a lectin with an affinity for mannose/glucose isolated from the Brazilian bean *C. brasiliensis*. ConBr shares primary carbohydrate recognition specificity with other legume plant lectins from the genus *Canavalia*, and the amino acid sequence displays 99% homology with ConA, the lectin of *Canavalia ensiformis* seeds [12,13]. Despite the structural similarity among these lectins, ConBr exerts biological effects different from those of ConA [14]. Therefore, further studies are needed to better understand the action of these lectins on different cell surfaces.

This work aimed to investigate the anthelmintic effect of *Canavalia brasiliensis* (ConBr) lectin against *H. contortus* and to evaluate a possible interaction of ConBr with glycans of this parasite by molecular docking.

2. Material and methods

2.1. Protein purification

Seeds from *C. brasiliensis* were ground to a fine powder in a coffee mill, and the soluble proteins were extracted at 25 °C by continuous stirring with 0.15 M NaCl [1:10 (w:v)] for 4 h, followed by centrifugation at 10,000 × *g* at 4 °C for 20 min. Protein purification was carried out by the affinity chromatography protocol, as previously described by Moreira and Cavada [15], using a Sephadex G-50 column (2 × 10 cm). This fraction (*C. brasiliensis* lectin - ConBr) was freeze-dried and purity-tested by SDS-PAGE [16].

2.2. Hemagglutination activity and inhibition assays

Hemagglutination assays were carried out as described elsewhere [17] using serial dilutions with rabbit erythrocytes, either native or treated with proteolytic enzymes (trypsin or papain). Results were expressed in hemagglutinating units (HU), with one HU being defined as the smallest amount (mg) of protein per mL capable of inducing visible agglutination. Lectin carbohydrate-binding specificity was defined as the smallest sugar concentration capable of fully inhibiting agglutination. Two-fold serial dilutions (initial concentration: 100 mM) of D-glucose, D-galactose, D-mannose, L-fucose, L-rhamnose, α-lactose, β-lactose and saccharose were prepared in 150 mM NaCl. Lectin (4 HU) was added to each dilution.

2.3. Molecular docking

The crystal structure of ConBr monomer (PDB code 3JU9) [18] and ConA monomer (PDB 1CVN) [19] were used for all docking simulations. The three-dimensional structure of the core trimannoside [Man (α1-3)]Man(α1-6)Man of the *H. contortus* glycan was drawn using the online software PRODRG [20]. Molecular docking analysis was performed with Molegro using the MolDock method [21]. MolDock is based on a search algorithm combining differential evolution with a cavity prediction algorithm. The program takes hydrogen bond directionality into account as an additional term in the docking scoring function. A re-ranking procedure was added to increase docking accuracy. A MolDock Score (MDS) was calculated using the scoring function. Grid resolution was 0.30 Å with radius of 15 Å. The search algorithm used was MolDock Optimizer with default settings. The number of runs was 10, and the maximum number of interactions was 2000. The population size and maximum number of poses were 200 and 10, respectively. Protein–ligand interaction energy was expressed in the form of the MDS in arbitrary units. A more negative value reflects a stronger interaction. The MDS was calculated with the following equation: MDS = E_{inter} + E_{intra}, where E_{inter} is the ligand–protein interaction energy [21].

$$E_{inter} = \sum_{i \in ligand} \sum_{j \in ligand} \left[E_{PLP}(r_{ij}) + 332.0 \frac{q_j\, q_j}{4r_{ij}^2} \right]$$

Polar interactions were analyzed with the CCP4 software CONTACT [22], adopting the cutoff distances of 3.2 Å. All figures and superposition were performed with the PyMOL program [23].

2.4. Biological assays

2.4.1. Obtaining nematodes

A strain of *H. contortus* was maintained in sheep which were fed with hay, 2% live weight of balanced ration (20% of protein), and water *ad libitum*. This experiment was approved by the Ethics Committee on Animal Experimentation of UFMA, Brazil under protocol number 23115.005443/2017-51.

Fresh feces from sheep artificially infected with *H. contortus* were macerated, washed with distilled water and passed through 1 mm, 105 µm, 55 µm and 25 µm graduated screens. The eggs were suspended in saturated saline and then washed with distilled water [24].

To obtain *H. contortus* L3 larvae, feces from lambs artificially infected were macerated, placed in glass beakers and incubated at 27 °C for 15 days. After that, the beakers were then filled with warm water, the contents poured into a Petri dish, the L3 larvae allowed to migrate, and storage set at 6 °C [25,26].

2.4.2. Egg hatch test

ConBr was diluted in PBS (pH 7.2) with six serial dilutions at 50% and 1.2 mg mL^{-1} as initial concentration. Approximately 100 eggs $well^{-1}$ were placed in 96-well plates with different concentrations (four replicates) and incubated for 48 h (27 °C, relative humidity > 80%). For the control, eggs were incubated with the same buffer used to dissolve ConBr. Eggs and larvae were counted in an inverted microscope [27].

2.4.3. Larval exsheathment test

ConBr was diluted in PBS (pH 7.2) with six serial dilutions at 50% and 1.2 mg mL^{-1} as initial concentration, followed by the addition of *H. contortus* L3 and incubation at 21 °C for 3 h. Initially, *H. contortus* larvae (L3) were subjected to the exsheathment process by contact with sodium hypochlorite solution (2%). After sieving, the larvae were centrifuged in distilled water for 5 min at 407 × *g*, and the supernatant was removed. The larvae were resuspended in distilled water and centrifuged. This process was repeated twice, until all sodium hypochlorite solution was removed. L3 exsheathment was counted. The same buffer used to dissolve ConBr was also used for the control [28].

2.4.4. Larval development test

H. contortus eggs were obtained, as previously described. One hundred eggs $well^{-1}$ (100 µL) were added to a 96-well plate and incubated at 27 °C for 24 h to obtain first-stage larvae (L1). Forty µL of solution containing *Escherichia coli* (autoclaved *E. coli*) 0.11 mg mL^{-1}, NaCl 2.24 mg mL^{-1}, yeast extract 2.8 mg mL^{-1}, amphotericin B (Sigma A2942) 0.018 mg mL^{-1} and 2.8% of Earle's solution (Sigma E7510) were added in all wells. ConBr was diluted in PBS (pH 7.2) with six serial dilutions at 50% and 0.5 mg mL^{-1} as initial concentration. Four replicates of each concentration were added in all wells and incubated at 27 °C for six days. After that, L1 and L3 were counted under an inverted microscope [29].

2.5. Data analysis

The data were initially transformed to Log(X) and normalized, followed by calculation of nonlinear regression to get the IC_{50} (50%