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NATURAL LECTIN ACTIVITY IN THE HAEMOLYMPH OF *PANSTRONGYLUS MEGISTUS* (HETEROPTERA: REDUVIIDAE)

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The haemolymph of Panstrongylus megistus *showed a natural lectin activity for a wide range of vertebrate erythrocytes. Agglutination was observed against all vertebrate erythrocytes tested (human ABO, duck, rabbit, mouse, sheep, chicken and cow). Cow erythrocytes showed the lowest titre. Concerning human erythrocytes, the lectin activity was similar in the types A^+, B^+ and AB^+ while the highest activity was observed in the type O^+. Determination of minimal inhibitory concentrations was carried out with human erythrocytes type O^+. Agglutination was inhibited by several carbohydrates (rhamnose, D-galatose, raffinose, D-lactose and D-fucose). Rhamnose was reported as the strongest inhibitor (0.78mM). The results suggest the presence of more than one lectin in the haemolymph of* P. megistus.

Key words: ***Panstrongylus megistus*** – haemolymph – lectin – carbohydrate specificity

The invertebrate humoral factors upon which most attention is focused are the "haemagglutinins", so called because of their ability to agglutinate vertebrate erythrocytes "in vitro" (Lackie, 1980). The specificity and the titres of these invertebrate agglutinins vary. These agglutinins are also termed lectins, a word proposed by Boyd & Shapleigh (1954), that means "choose" or "pick out". The lectins are sugar-binding proteins or glycoproteins of non-immune origin, with more than one carbohydrate binding site, which agglutinate cells and/or precipitate glycoconjugates (Goldsteins et al., 1980; Dixon, 1981).

The occurrence of lectins in insects has been reviewed by Yeaton (1980) and, although the biological and physiological functions of insect lectins are still unclear, proposed roles include their involvement in receptor recognition and in defense mechanisms (Rögener & Uhlenbruck, 1984).

Many experiments have been carried out to characterize the lectin activity in the haemolymph of insects and to determine the binding sites of these lectins, by means of inhibition assays (Scott, 1971; Komano et al., 1980; Lackie, 1981; Jurenka et al., 1982; Hapner, 1983). Naturally occurring lectins against trypanosomatid flagellates have been found in the haemolymph of locusts, *Schistocerca gregaria*, and cockroaches, *Periplaneta americana* (Ingram et al., 1984). The agglutination of erythrocytes and *Leishmania* parasites by gut extracts of *Phlebotomus papatasi* was reported by Wallbanks et al. (1986). In triatomines, Pereira et al. (1981) have reported that lectin activities of distinct carbohydrate specificities were present in the crop, midgut, and haemolymph of *Rhodnius prolixus*, and that each lectin is highly specific in interacting with developmental stages of *Trypanosoma cruzi.*

This work investigates the lectin activity in the haemolymph of *Panstrongylus megistus* by testing the activity against erythrocytes from different species of vertebrate, and by carring out inhibition studies to determine its binding specificities.

MATERIALS AND METHODS

Insects – Fifth instar larvae *P. megistus*, six days after a blood meal in guinea pig, were used. The insects were reared in an incubator at 25 °C ± 2 and R. H. of 70% ± 5 in the insectary of the Centro de Pesquisas Aggeu Magalhães – FIOCRUZ, Recife, Brazil.

Haemolymph – Haemolymph was collected with a capillary pipette by excising the mesothoracic leg, centrifuged for 60 min/750 x g/4 °C to remove haemocytes, and the supernatant stored at −20 °C. The haemolymph of

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60 insects was pooled to carry out inhibitory studies and to verify the haemagglutinating activity. In order to observe the influence of sex in this last point the haemolymph of 10 males and 10 females was used.

To investigate the influence of haemocyte lysis on the hemagglutinating activity the haemolymph of 30 insects was pooled and the following procedures were carried out: i) 0.6 ml were filtered in 0.2 μm Millipore membrane, ii) 0.6 ml were freeze-thawed three times, centrifuged, and the supernatant assayed for hemagglutinating activity. The supernatant and sediment were stained with Giemsa.

Agglutination assay – Agglutination assay was carried out in microtitre U plates by two-fold serial dilution of 20 μl haemolymph in 0.15 M NaCl solution. Human and other vertebrate erythrocytes (duck, rabbit, mouse, sheep, chicken and cow) were washed five times prior to use and resuspended in the same solution to give a final 1% (v/v) cell suspension. All experiments were performed at room temperature. Results were read macroscopically and titres recorded as the reciprocal of the last haemolymph dilution that agglutinated erythrocytes. To observe the influence of the sex and the haemocyte lysis, haemagglutinating activity was performed using O^+ erythrocytes.

Inhibition of haemagglutination – Inhibition tests were performed using O^+ human erythrocytes. 20 μl of the haemolymph was diluted in 20 μl of 0.15 M NaCl containing several concentrations of inhibitor (200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 mM). After being submitted to double dilutions it was incubated for 30 min, at room temperature, after that 1% (v/v) cell suspension was added. Carbohydrates tested were: N-acetyl-D-mannosamine; N-acetyl-D-glucosamine; β-metyl-D-arabinopiranoside; methyl-α-D-manoside; L-fucose; D-fucose; D-xilose; D-galactose; D-lactose; L-sorbose; rhamnose; raffinose; maltose and glucose.

RESULTS

Agglutination assay – Haemagglutination titre related to sex is shown in Table I. The results show that haemagglutinating activity is present in the haemolymph of both male and female insects, and this activity is similar.

TABLE I

Haemolymph haemagglutination titre in relation to sex of *Panstrongylus megistus*

Sex	Insect number	Physiological stage*	Developmental stage	Titre
Male	10	6	5th instar	2,048
Female	10	6	5th instar	4,096

* Days after meal.

All human and animal erythrocytes tested were agglutinated by haemolymph of *P. megistus*. However, some types of cells were agglutinated more strongly than others (Table II). Concerning human erythrocytes, lectin activity was the same for the types A^+, B^+ and AB^+ while the highest activity was obtained with the type O^+ (4.096). Agglutination titre among animal cells presented no appreciable difference excluding cow that showed the lowest titre.

TABLE II

Haemagglutination activity in the haemolymph of fifth instar larvae of *Panstrongylus megistus*

Erythrocytes		Titre
Human Rh^+	O	4,096
	B	1,024
	AB	1,024
	A	1,024
Duck		1,024
Rabbit		1,024
Mouse		1,024
Sheep		256
Chicken		256
Cow		64

No significant difference was observed in haemagglutinating activity with the haemolymph filtered and lysed haemocytes as well as control (Table III).

TABLE III

Hemagglutinating activity according to cell integrity

Haemolymph	Titre
Filtered	2,048
Lysed haemocyte	2,048

Inhibition tests – Only five out of the fourteen tested carbohydrates inhibited agglutination of human erythrocyte O^+. Minimal carbohydrates concentrations inhibition of *P. megistus* lectin are summarized in Table IV.

TABLE IV

Inhibition by carbohydrates of agglutination of human erythrocyte type O^+ with *Panstrongylus megistus* lectins

Inhibitor	Minimal inhibitory concentration (mM)
N-Acetyl-D-manosamine	–
N-Acetyl-D-glucosamine	–
β-Metyl-D-arabinopiranoside	–
Metyl-α-D-manoside	–
L-fucose	–
D-fucose	25.0
D-xylose	–
D-galactose	25.0
D-lactose	6.25
L-sorbose	–
Rhamnose	0.78
Raffinose	6.25
Maltose	–
Glucose	–

– No inhibition.

DISCUSSION

It was observed a natural lectin activity in the haemolymph of *P. megistus*. Heamolymph agglutinated all types of erythrocytes tested showing a variation in the agglutination titre suggesting the presence of a common polysaccharide on the surface of these erythrocytes.

Pereira et al. (1981) showed that the lectin present in the haemolymph of *R. prolixus* weakly agglutinated rabbit and dog erythrocytes. After neuraminidase treatment the agglutination titre became higher. However, other erythrocytes used (human, opossum, sheep, guinea pig, mouse, hamster and goose) were not agglutinated either before of after enzymatic treatment. Definitely the lectins of haemolymph of *P. megistus* are different from those found in *R. prolixus* since the haemolymph of the former agglutinated diferent types of erythrocytes even without enzymatic treatment. The haemolymph of *P. megistus* showed higher activity when compared to the haemolymph of *R. prolixus* and other studied insects (Scott, 1971; Lackie, 1981; Jurenka et al., 1982; Hapner, 1983). This high agglutination effect of *P. megistus* haemolymph naturally occurs even in the absence of injury, contrary to what was reported by Komano et al. (1980) in *Sarcophaga peregrina.*

Experiments for determination of minimal inhibitory concentrations were performed with O^+ human erythrocytes in order to define the carbohydrate binding specificity to the lectins present in the haemolymph of *P. megistus*. According to definition of specificity in lectins by Goldstein et al. (1980), the haemolymph of *P. megistus* contains lectins with specificity to rhamnose, since it was the best inhibitoring carbohydrate (0.78 mM). Inhibition was also observed in the presence of other saccharides (D-lactose, raffinose, D-galactose and D-fucose) but in higher concentrations, while it did not occur in the presence of β-methyl-D-arabinopiranoside, N-acetyl-D-mannosamine, N-acetyl-D-glucosamine, α-methyl-D-manoside, L-fucose, D-xylose, L-sorbose, maltose and glucose. This may suggest that the lectins present in the haemolymph of *P. megistus* do not have recognition sites for these structures.

The results reported above lead us to conclude that the haemolymph of *P. megistus* shows natural lectin activity; it was not specific toward the vertebrate erythrocytes studied. This activity is inhibited by several carbohydrates, suggesting the presence of more than one lectin. In addition this hemagglutinating activity is present is both sexes and the lysed cells did not influence such an activity.

Additional molecular characterization, including isolation of lectins, is required to confirm these findings. Isolation and molecular characterization of *P. megistus* lectins is in progress in our laboratory.

RESUMO

Atividade lectínica na hemolinfa de *Panstrongylus megistus* (Heteroptera: Reduviidae) – A hemolinfa de *Panstrongylus megistus* mostrou uma atividade lectínica natural para eritrócitos de vários vertebrados e não mostrou especificidade para os diversos tipos de eritrócitos testados (humano ABO, pato, coelho, camundongo, carneiro, galinha e boi). Com relação aos eritrócitos humanos a atividade lectínica foi similar nos tipos A^+, B^+ e AB^+ enquanto a ativi-

dade mais alta foi observada no tipo O^+. O título de aglutinação entre eritrócitos animais não mostrou diferença apreciável, excluindo eritrócitos de boi, que apresentaram o título mais baixo. A determinação da concentração mínima de inibição foi realizada com eritrócitos humanos O^+. A aglutinação foi inibida por vários carboidratos (ramnose, D-galactose, rafinose, D-lactose e D-fucose). A ramnose foi o inibidor mais potente (0,78 mM). Os resultados sugerem a presença de mais de uma lectina na hemolinfa de *P. megistus*.

Palavras-chave: *Panstrongylus megistus* – hemolinfa – lectina – carboidrato-específico

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