

NATURALLY OCCURRING LECTINS IN THE HAEMOLYMPH OF *PANSTRONGYLUS MEGISTUS* (HEMIPTERA: REDUVIIDAE)

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Agglutinins or lectins are ubiquitous molecules that occur throughout plants, animals and microorganisms. They possess carbohydrate binding sites and usually bind to erythrocytes, spermatozoa, bacteria and other cells causing their respective agglutination or precipitation. Most Arthropoda agglutinins that have been studied are humoral components of the haemolymph. However, they are also present in other tissues (review: Stebbins & Harpner, 1986, p. 463-491. In: Gupta, A. P. ed. *Hemocytic and humoral immunity in Arthropods*. John Wiley & Sons). In Arthropoda they are suspected of having recognitory and immune-like roles associated with defense and tissue maintenance systems (Stebbins & Harpner, 1986, loc. cit.).

We report in the present paper, the detection, by haemagglutination assays, of naturally occurring agglutinins or lectin in the haemolymph of 4th and 5th instar nymphs of *Panstrongylus megistus* from Sta Catarina Island. For these assays, we have used fresh human red blood cells (HRBC) of the ABO system. The haemagglutination experiments revealed the presence of anti-A, anti-B and anti-H lectin activities (La-A, La-B and La-H) in the haemolymph. Haemolymph produced a very characteristic agglutination pattern of B erythrocytes forming very large cell clumps. On the other hand, the haemagglutination pattern of A and O erythrocytes by haemolymph was quite different, consisting of thin and small erythrocyte aggregates, with powdery aspect. The agglutination pattern of B erythrocytes was, thus, clearly distinguishable, from the agglutination of the other two human red blood cell types.

To titrate the lectins, we used 50 μ l of a 10% suspension of HRBC in phosphate buffer

saline, 0.01 M, pH 7.2 (PBS) and 50 μ l of two fold serial dilutions of haemolymph in micro-titrator plates, following standard methods. For inhibition of agglutination, the haemolymph was heated at 57 °C and 70 °C for 10 min. The haemolymph was also treated with EDTA at different concentrations (from 0.02 M to 0.2 M). To define the specificity of agglutination, the haemolymph was incubated with different sugar concentrations, corresponding to the different human erythrocytes of ABO system (N-acetyl-D-galactosamine; D-galactose and L-fucose).

For the analysis, haemolymph was always collected from a severed leg or antenna in Eppendorf tubes. The hemocyte fraction was discarded by centrifugation.

It can be observed from the results (Table) that La-A and La-H are thermolabile, similarly to other Arthropoda lectins (Amirante, 1986, p. 359-380. In: Gupta, A. P. ed. *Hemocytic and humoral immunity in Arthropods*. John Wiley & Sons). On the other hand, even when heated at 70 °C, La-B still promotes erythrocyte agglutination. La-B is, thus, not thermolabile. In this respect, it behaves differently from agglutinins described for other insects.

The haemolymph lectins of *P. megistus* were not affected by EDTA (Table), even at high concentrations (0.2 M). However, the agglutinins of other insects are generally $\text{Ca}^{++}/\text{Mg}^{++}$ dependent (Rowley et al., 1986, p. 381-406. In: Gupta, A. P. ed. *Hemocytic and humoral immunity in arthropods*. John Wiley & Sons) and low concentrations of EDTA (0.02 M) are sufficient to inhibit agglutination.

Haemolymph of *P. megistus* strongly agglutinates trypomastigote forms of *Trypanosoma cruzi* (SC-28 strain), obtained from blood of infected mice in opposition to what occurs in *Rhodnius prolixus*. In this reduviid, the lectins of haemolymph agglutinated only epimastigote forms of *T. cruzi*, Y and Cl strain (Pereira et al.,

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TABLE

Titration of anti-A (La-A), anti-B (La-B) and anti-H HRBC (La-H) lectins of hemolymph of *Panstrongylus megistus* by haemagglutination assays

Type	Titration	Inhibition by temperature		Inhibition by EDTA 0.2 M	Inhibition by sugars
		57 °C	70 °C		
La-A	64	4	0	64	GalNAc 9 mM
La-B	256	256	256	256	Gal 9 mM
La-H	256	4	0	256	Fuc 1 mM

Temperature, EDTA and different sugars were used as agglutination inhibitors. The numbers represent the reciprocal of the highest dilution causing agglutination. The different sugars inhibit agglutination in different concentrations. GalNAc – N-Acetyl D-galactosamine; Gal – D-galactose; Fuc – L-fucose.

1981, *Science*, 211: 597-600). When drops of *P. megistus* haemolymph are placed together with drops of trypomastigote forms of *T. cruzi*, maintained in LIT medium, an intense parasite agglutination can be observed under phase contrast microscopy. In control experiments, where haemolymph is replaced by PBS, agglutination does not occur. *P. megistus* haemolymph certainly must contain lectins which bind to *T. cruzi* surface. The presence of galactose and N-acetyl-galactosamine residues on *T. cruzi* surface has already been reported (Zimmermann et al., 1987, *Parasitol. Res.*, 74: 11-17). It can, thus, be suggested that La-B and La-A may be related to parasite agglutination in haemolymph.

As we mentioned before, in *R. prolixus*, Pereira et al., 1981 (loc. cit.), reported that the haemolymph lectins only agglutinated epimastigote and not trypomastigote forms of *T. cruzi* (Y and Cl strains) isolated from culture medium or purified from the blood of infected mice. These authors claim that the interaction of the insect lectins with the trypanosome is stage-specific. They reported the presence of lectins of different activities in the haemolymph of the reduviid *R. prolixus*. Haemolymph lectins of *R. prolixus* were preferentially inhibited by saccharides of the D-galactose and N-acetyl-D-galactosamine configuration similarly to what happened with *P. megistus* (Table).

Pereira et al., 1981 (loc. cit.) were more interested in the selective agglutination of epimastigote forms of *T. cruzi* by lectin of different sources of *R. prolixus* that in the role of haemolymph lectins in humoral immunity.

Knowing the results obtained by Pereira et al., 1981 (loc. cit.) in *R. prolixus*, Ibrahim et al., 1984 (*Tropenmed. Parasit.*, 35: 151-156) performed similar studies with the dipteran *Glossina austeni*, vector of African trypanosomes. They observed that, opposedly to what occurs with *R. prolixus* and *P. megistus* the haemolymph lectins of *G. austeni* were very poorly inhibited by D-galactose and N-acetyl-D-galactosamine. Furthermore, these haemolymph lectins did not agglutinate procyclic forms of *Trypanosoma brucei*, although these parasites were agglutinated by midgut and hindgut extracts. However, in other dipterans such as *Sarcophaga bullata* and other calliphorid flies, D-galactose and its derivatives inhibited mainly the haemagglutination of human B erythrocytes by haemolymph (Stynen et al., 1985, *Comp. Biochem. Physiol.*, 81B: 171-175), similarly to what happens in *P. megistus* and *R. prolixus*.

In some Arthropoda, lectins can be found on hemocyte surface (Amirante, 1986, loc. cit.) as it occurs with the vertebrate immunoglobulins on B-lymphocytes membranes. In order to detect the presence of lectins on the surface of *P. megistus* hemocytes, we promoted the adhesion of hemocytes on coverslips. These, were maintained for 1 h in a small petri dish, containing a physiological Carlson solution for insects. After this period, the coverslips were washed with the same saline solution and the adhered hemocytes where, covered with a 0.5% suspension of HRBC for 1 h. After this time, they were carefully washed with PBS and the presence of rosette-like figures was investigated

using the phase contrast microscope. Only B erythrocytes originated scarce rosette-like formations with hemocytes. These experiments may suggest that L-A and L-H are not bound to hemocyte surface while La-B may be present in small concentrations. However, further experiments are needed to confirm these findings.

Our results show that the lectins of haemolymph of *P. megistus* agglutinate trypomastigote forms of *T. cruzi* in contrast to what occurs with *R. prolixus* (Pereira et al., 1981 loc.

cit.). Anti-B lectin of *P. megistus* seems to be a distinct type when compared to La-A and La-H. The differences are related to agglutination pattern, thermolability and rosette formation. Further experiments will be performed in order to reach more conclusive results.

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