EXPLORATORY STUDY OF ISOENZYMES IN THE HEMOLYMPH OF TRIATOMINES, VECTORS OF CHAGAS. II - *Panstrongylus megistus* (BURMEISTER, 1835)

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RESUMO

Estudo exploratório de isoenzimas na hemolinfa de triatomíneos vetores da doença de chagas. II - *Panstrongylus megistus* (Burmeister, 1835)

Caracterização dos perfis eletroforéticos de isoenzimas (Alcool Desidrogenase (ADH) E.C. nº 1.1.1.1; Galactose Desidrogenase, (GAL-DH) E.C. nº 1.1.1.48; Sorbitol Desidrogenase (SOR-DH) E.C. nº 1.1.1.14; Transaminase Glutâmica Oxaloacética (GOT) E.C. nº2.6.1.1; Esterase (EST) E.C. nº 3.1.1.1; Leucina Aminopeptidase (LAP) E.C. nº 3.4.11.1; Octanol Desidrogenase (ODH) E.C. nº 1.1.1.73) presentes na hemolinfa de *Panstrongylus megistus* (Burmeister, 1835).

INTRODUCTION

Triatomines comprise an extremely large number of species, all hematophagous, which are found in different ecological environments, and some of which utilize a wide range of hosts. (BARRETO, SIQUEIRA & CORREA, 1963; BARRETO, 1964, 1967; FORATTINI & SILVA, 1970; LENT & WIGODZINSKY, 1970).

The reasons why some species are more ecologically restricted while others are more generally distributed depend on many factors; among them are nutritional requirements and the ability to utilize the available food resources in a more effective way. In this respect, enzymes play an important role in food metabolism (LWOFF & NICOLE, 1945; JOHNSON, 1973; ALMEIDA *et alii*, 1979b). In general, an increase in variability of metabolic pathways corresponds to a better level of utilization of food (ALMEIDA *et alli*, 1979 a, b).

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A comparative study of the electrophoretic profiles of some isoenzymes present in the hemolymph of triatomines, having digestive and intermediary metabolic functions (ALMEIDA, in press, a, b) was undertaken in order to detect the variability of these and to determine their implication in the level of ecological specialization or generalization of these species in terms of feeding.

In the present study, we investigated octanol dehydrogenase (ODH), alcohol dehydrogenase (ADH), galactose dehydrogenase (GAL. DH), sorbitol dehydrogenase (SOR. DH), glutamic oxaloacetic transaminase (GOT), leucine amino peptidase (LAP), alpha and beta esterase (EST) and proteins.

MATERIAL AND METHODS

Nymphs and adults of *Panstrongylus megistus* (Burmeister, 1835) were used. These hematophagous hemiptera were separated into four colonies and allowed to feed on four types of hosts: *Cavia porcellus*, *Mus musculus*, *Columba livia* and *Gallus gallus* selected for being probably part of the innumerable natural sources of food for wild and house-peripheral triatominae. Variation of the physical factors during the insect growth phase throught the experiment was as follows: temperature $\bar{X}$: 30°C; $\bar{X}$max: 33°C; $\bar{X}$min: 26°C; relative humidity $\bar{X}$: 75%, $\bar{X}$max: 80%, $\bar{X}$min: 70%. Alternate periods of 12 hours of light and 12 hours of dark were provided. The enzymes and the proteins present in the hemolymph of *P. megistus* were detected after electrophoresis in a discontinuous Tris-citrate I system (SHAW, 1969), with 0.3M borate, pH 8.2, as the electrode buffer and 0.076M Tris, containing 0.005M citric acid, pH 8.7, as the gel buffer. Hemolymph (5µℓ) was collected by inserting a graduated pipette into the thigh of the triatomines and immediately transfered to small pieces (3x6mm) of Whatmann 3mm filter paper and applied directly to sample groves in the gel, at 5°C (ALMEIDA *et alii*, 1978).

Electrophoresis was carried out for 4 hours at 5°C, 400V, 80mA, or until the front had migrated 9cm. After electrophoresis, the gel was cut into three sections, incubated and developed at 37°C with specific substrates and dyes. The gel was then washed in water, fixed with a 5:5:1 solution of methanol, water and acetic acid, dried and wrapped in plastic film, and zymogram measurements were made (POULIK, 1957).

The standard reference band of *Oncopeltus fasciatus* Dallas, 1852 (OF-1) was used to identify and calculate the RF (degree of electrophoretic mobility with reference to a known standard) of the isoenzyme bands (NARANG & KITZMILLER, 1971) of the triatomines, as follows:

$$RF = \frac{\text{Distance travelled by the isoenzyme band}}{\text{Distance travelled by the OF-1 esterase band}}$$

So, for instance, the RF values detected for ODH should be 5 (ODH-5) and 25 (ODH-25).

RESULTS

Only two zymograms patterns were found for the ODH systems after analysis of 20 males, 20 females and 20 nymphs (Fig. 1). The ODH-5 band was quite homogeneous; however, the ODH-25 band was wider in most individuals, appearing to consist of 3 or more bands so close to each other that the diffusion and incomplete separation of the components made them appear as a single band.

The same sample was used for ADH detections and two electrophoretic patterns were also found for this system (Fig. 1). The ADH-25 band also seemed to consist of at least three bands. The bands could probably be separated using a more sensitive technique, such as acrylamide gel electrophoresis.

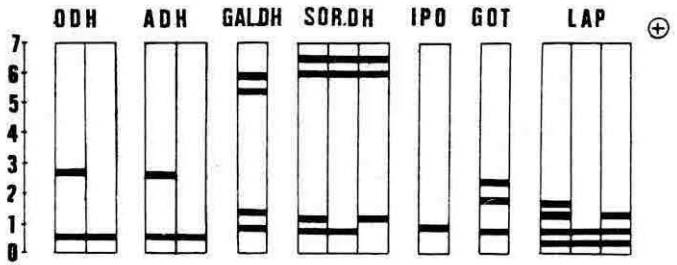

FIG. 1- ODH, ADH, GAL.DH, SOR.DH, IPO, GOT and LAP -electrophoretic profiles. The full bands represent the isoenzyme bands and the cm scale indicates the distance run by the bands for 4 hours.

Twenty males and 20 females were used for GAL-DH detection. Only one pattern with four bands was obtained: GAL.DH-8, GAL.DH-13, GAL.DH-55 and GAL.DH-60 (Fig.1).

Twenty males and 20 females were used for SOR.DH detection. Three types of patterns were obtained for both sexes, containing the following bands: SOR.DH-8, SOR.DH-13, SOR.DH-60 and SOR.DH-65.

A total of 60 insects (20 males, 20 females and 20 larvae) was used for IPO. A single band was detected and was present in all individuals (Fig. 1).

Sixty individuals were also used for detection of the GOT system (20 males, 20 females and 20 5th-instar nymphs). Only one profile was detected, with three bands: GOT-8, GOT-16 and GOT-22 (Fig.1).

For the characterization of leucine aminopeptidase activi-

ty (LAP), 20 males and 20 females were studied. Only two patterns were found, with two or four bands both in males and females: LAP-4, LAP-7, LAP-12 and LAP-17 (Fig. 1).

For alpha and beta esterase, seven types of profiles were detected, having a maximum of 9 bands and a minimum of seven. The following bands were detected: EST-12, EST-19, EST-33, EST-36, EST-40, EST-44 and EST-55. Five bands (EST-12, EST-19, EST-24, EST-33 and EST-55) were detected for all of the 62 insects studied (Fig.2). Total soluble proteins were studied in 40 *P. megistus* specimens. A total of five different profiles was found, though the males had more diversity (four profiles) than the females (three profiles). At least 15 bands were present in the zymograms (P-2, P-5, P-10, P-14, P-18, P-22, P-29, P-32, P-40, P-45, P-51, P-55 and P-60), 12 of which were present in all individuals studied (Fig. 3).

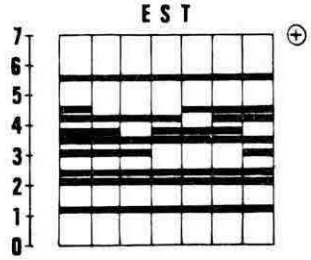

FIG. 2- Electrophoretic profiles of esterase in *Panstrongylus megistus* (Burmeister, 1835) hemolymph.

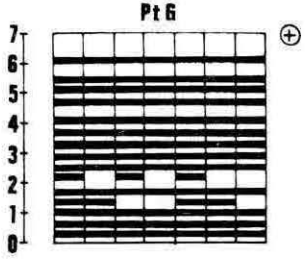

FIG. 3- Electrophoretic profiles of total protein in *Panstrongylus megistus* (Burmeister;1835) hemolymph.

DISCUSSION

When more than one electrophoretic pattern was detected, the individuals having a higher amount of bands were normally more numerous, possibly because a larger number of bands represents in general the heterozygote (in the case of polymorphic loci), or because a larger number of bands means a larger number of enzymes produced by different loci (in the case of nonpolymorphic loci), which might confer a greater amplitude of metabolic adaptation to insects having these genotypes (ALMEIDA, 1981).

P. megistus might have two loci in the ADH system: the ADH

-5 locus, which may be monomorphic and produce only one band, and locus ADH-25, which may be polyellelic (with dimeric alleles), with the homozygote producing one band and the heterozygote producing three bands. The same seems to occur in the ODH system, i.e., two loci may be involved: ODH-5 (monomorphic) and ODH-25 (polymorphic with dimeric alleles), with the homozygotes producing one band and the heterozygotes producing three bands. To confirm this hypothesis, a more sensitive technique providing better band separation is needed.

It must not be simply a coincidence that in the same animal species enzymes of the same group (dehydrogenase-type oxidoreductases) but belonging to different enzyme systems have the same electrophoretic mobility and identical characteristics (ADH-5 and ODH-5 with monomorphism, and ADH-25 and ODH-25 with dimeric polymorphism). It is likely that only two loci, DH-5 and DH-25, produce enzymes that catalyze the same type of reaction (dehydrogenation) with several structurally related substrates, i.e., reactions with other substrates can occur as long as these substrates are present at high concentration. This is because the occurrence of all possible reactions in living organisms depends in part on the relative concentration of alternate substrates in the cell and on their relative affinity for an enzyme.

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ABSTRACT

The caracterization of the electrophoretic profiles of isoenzymes (Alcohol Dehydrogenase (ADH) E.C. nº 1.1.1.1, Galactose Dehydrogenase (GAL-DH) E.C. nº 1.1.1.48, Sorbitol Dehydrogenase (SOR-DH) E.C. nº 1.1.1.14, Glutamic Oxaloacetic Transaminase (GOT) E.C. nº 2.6.1.1, Esterase (EST) E.C. nº 3.1.1.1, Leucine Aminopeptidase (LAP) E.C. nº 3.4.11.1, Octanol Dehydrogenase (ODH) E.C. nº 1.1.1.73) present in the hemolymph of *Panstrongylus megistus* (Burmeister, 1835).