

**BIOLOGY OF AMAZONIAN ANOPHELINES. XIX.
 α -GLYCEROPHOSPHATE DEHYDROGENASE IN
Anopheles nuneztovari: (DIPTERA: CULICIDAE) ONTOGENY AND
GENETIC VARIATION**

Vera Margarete Scarpassa¹ and Wanderli Pedro Tadei^{1,2}

ABSTRACT

During the ontogenetic development of *Anopheles nuneztovari*, the electrophoretic profiles of α -glycerophosphate dehydrogenase permitted the diagnosis of an activity region, near the origin, which increased in intensity towards the adult stage. From the first instar larvae to the 4-hour-old pupae there is low enzymatic activity. Intense activity is seen in 12-hour-old pupae and continues to the recently emerged adult. The profile of the larval stage showed a slight difference in migration compared to the pupal and adult profiles. This allowed us to raise the hypothesis of the existence of two forms of the enzyme, products of independent loci, or, more probably and as has been widely demonstrated in the genus *Drosophila*, the product of one locus, but with post-synthesis modification based on a polypeptide. The more intense activity in the adult stage may indicate that the enzyme presents greater concentrations in the thorax, having functions related to flight activity. Also correlated to flight activity was the low level of allele variation.

INTRODUCTION

α -glycerophosphate dehydrogenase (α -GPDH, E.C.1.1.1.8) has been shown to be an ideal system for the study of the changes in genic expression during development in insects. One of the reasons for these changes is attributed to the importance of the enzyme in the flight mechanism in insects (MacDonald and Avise, 1976; Collier *et al.*,

1 Coordenação de Pesquisa em Ciências da Saúde, INPA, Caixa Postal 478, 69083-000 Manaus, AM, Brasil.
Send correspondence to V.M.S.

2 UNESP, São José do Rio Preto, SP, Brasil.

1976; Bewley and Miller, 1979; Sullivan *et al.*, 1983). In the thorax, particularly in the thoracic muscle, two enzymes participate actively in the α -glycerophosphate cycle: (1) α -GPDH, which is cytoplasmic, soluble, and dependent on NAD^+ , catalyzes the reduction of the dihydroxyacetone phosphate into α -glycerophosphate. This last substance penetrates easily into the interior of the mitochondria. (2) α -Glycerophosphate oxidase (α -GPO), which is mitochondrial, and independent of NAD^+ , converts α -glycerophosphate to dehydroxyacetone phosphate. The electrons liberated in this reaction go to the respiratory cycle of the sarcosome for production of ATP which liberates the energy necessary for flight (Sacktor and Dick, 1962; O'Brien and MacIntyre, 1972a; Collier *et al.*, 1976; Connors and Curtsinger, 1986; Davis and MacIntyre, 1988).

Detailed studies of α -GPDH have been carried out on *Drosophila*, with emphasis on aspects of ontogeny, patterns of genic expression in specific tissues, optimum pH, kinetic parameters, metabolic function and hereditary mechanisms (Rechsteiner, 1970; O'Brien and MacIntyre, 1972a; Miller *et al.*, 1975; MacDonald and Avise, 1976; Bewley and Lucchesi, 1976; Bewley, 1978; Bewley and Miller, 1979; Sullivan *et al.*, 1983; Laurie-Ahlberg *et al.*, 1985; Gibson *et al.*, 1986; Loreto and Oliveira, 1988). In *Drosophila melanogaster*, the activity of α -GPDH is associated with the appearance of three enzymes during development (Bewley and Miller, 1979). These authors proposed that all are products of a single structural gene and that the differences in the electrophoretic mobility are due to post translational modifications (Bewley and Miller, 1979; Niesel *et al.*, 1982). In Culicidae, different patterns of the genic expression of α -GPDH were observed during ontogenesis. Three isozymes were detected during development in *Anopheles albimanus* (Ved Brat and Whitt, 1974). In *Culex pipiens* (Pasteur and Stordeur, 1976) and *Aedes albopictus* (Tadano, 1984) only a single region of strong intensity was detected in the adult stage. Mukiyama (1980) observed two isozymes of α -GPDH in *Aedes aegypti*: α -GPDH1, present in larval and pupal stages, and α -GPDH2, specific to adults.

We studied the patterns of genic expression of α -GPDH in different stages of *Anopheles nuneztovari*, with starch gel electrophoresis and analyzed the genetic mechanisms of this system.

MATERIAL AND METHODS

Samples of *A. nuneztovari* were obtained at a site located on the right margin of the reservoir of the Tucuruí hydroelectric complex (Base 4), State of Pará, Brazil. The females, captured in nature, were separated individually for oviposition. After the eggs hatched, the larvae of each clutch were maintained in an insectarium at $26 \pm 1^\circ\text{C}$ until the adult stage was reached, following the procedure described by Scarpassa (1988) and Scarpassa and Tadei (1990).

Electrophoretic analyses were carried out on all the development stages. The 1st-, 2nd- and 3rd-instar larvae were homogenized in pools of 80, 40 and 10 larvae, respectively. The samples were individual for the 4th-instar larvae, pupae and adults. The 4th-instar larvae were separated into three phases: young, intermediary and old. Pupae were also separated into three groups: 4-, 12- and 24-hour old pupae.

Preparation of the samples for analyses followed the procedures described by Scarpassa *et al.* (1992). The support employed was starch gel (12.5%) in a horizontal system. Tris-phosphate buffer, pH 7.4, was used (Machado, 1986). The gels were submitted to a electric potential of 2.6 V/cm for 16 hours at 4°C and stained in a mixture containing 440 mg D.L. α -glycerophosphate substrate, 15 ml 0.06 M Tris-HCl buffer, pH 8.0, 14 mg NAD, 0.7 ml MTT, 0.5 ml PMS and 15 ml 2% agar in distilled water (Harris and Hopkinson, 1976).

RESULTS

The electrophoretic profiles of α -GPDH obtained during development are shown in Figure 1. For the four larval stages and 4-hour old pupae, a band of weak activity which increased gradually in the 12-hour pupae and became intense in the 24-hour pupae and newly emerged adults was observed. The activity was even greater in the adults when compared to 24-hour pupae. Figure 1 also shows that a band detected in the larval stages had a slight difference in mobility compared to the pupal and adult stages. The characteristic band of these last stages had a more anodic position in the gel, migrating an average of 1-2 mm compared to the larval band.

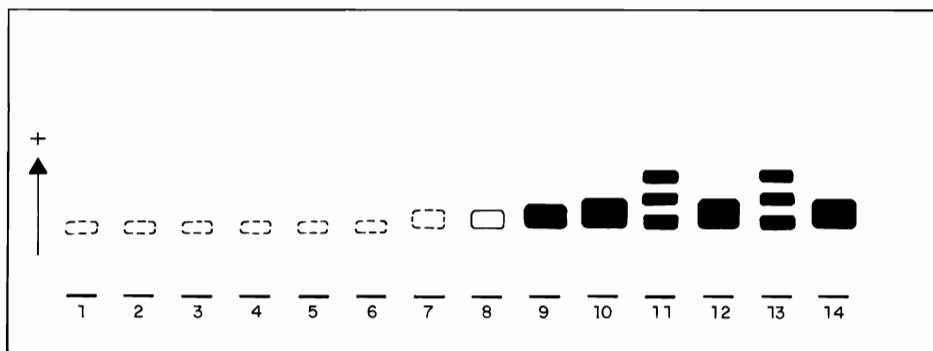


Figure 1 - Electrophoretic profiles, showing the increase in α -GPDH activity during the development of *A. nuneztovari*. Samples: (1) 1st instar larvae, (2) 2nd instar larvae, (3) 3rd instar larvae, (4) young 4th instar larvae, (5) intermediate 4th instar larvae, (6) old 4th instar larvae, (7) 4-hour pupa, (8) 12-hour pupa, (9) 24-hour pupa, (10-14) recently emerged adults.

Figure 2 is a photograph of the gel which shows an electrophoretic profile of the newly emerged adults.

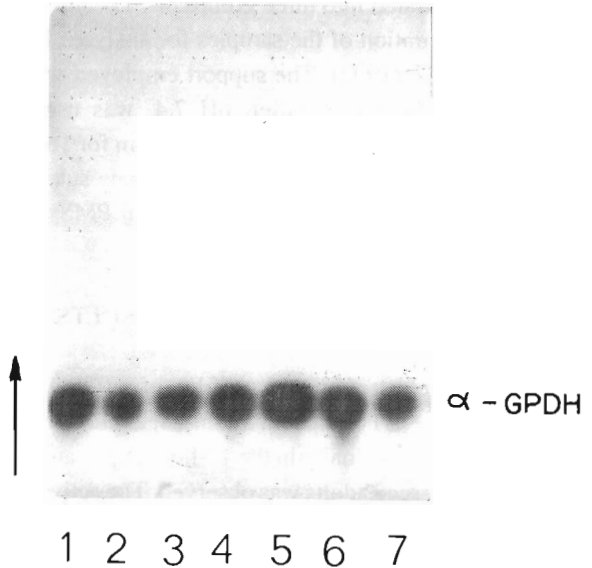


Figure 2 - Electrophoretic profiles of α -GPDH in recently emerged adults.

Allelic variation were detected at the α -GPDH locus with two distinct phenotypes on the gel. Of the 50 individuals analyzed for genic frequency of alleles (Table I), 47 showed the α -GPDH S/ α -GPDH S phenotype, three showed the α -GPDH F/ α -GPDH S phenotype and none showed the α -GPDH F/ α -GPDH F phenotype. These phenotypes indicate the existence of two alleles of codominant action which were designated according to their relative mobility on the gel, α -GPDH*F and α -GPDH*S. The heterozygous individuals presented a pattern with three bands, (Figure 1, samples 11 and 13), suggesting that the α -GPDH enzyme has a dimeric structure. The α -GPDH*S allele was the most frequent in the population (0.970), and the α -GPDH*F allele was much rarer - 0.030 (Table I). The chi-squared value was not significant for the observed and expected frequencies, indicating that the population is in Hardy-Weinberg equilibrium.

DISCUSSION

The genic expression of α -GPDH during the development of *A. nuneztovari* showed that the activity of this enzyme increases during the life cycle of the insect up to

Table I - Distribution of observed and expected frequencies and the chi-square values for the α -GPDH locus of *A. nuneztovari*. DF=1.

Locus	Class Genotípica	Obs.	Esp.	Frequency of alleles		χ^2 H-W
				α -GPDH*F	α -GPDH*S	
	α -GPDH*F/ α -GPDH*F	0	0.045	0.030	0.970	0.048
α -GPDH	α -GPDH*F/ α -GPDH*S	3	2.910			
	α -GPDH*S/ α -GPDH*S	47	47.045			
	N	50				

adult emergence. Similar patterns have been observed in other species of insects, such as *A. albimanus* (Ved Brat and Whitt, 1974), *C. pipiens* (Pasteur and Stordeur, 1976), *A. aegypti* (Mukiama, 1980), *A. albopictus* (Tadano, 1984), *Paratheresia claripalpis* (Marin and Mestriner, 1985) and *Spodoptera frugiperda* (Lima and Contel, 1990).

The literature discusses a complex mechanism of the genic expression of α -GPDH, including genic duplication and the action of regulator genes in the process. Experiments made with the genus *Drosophila* have also shown evidence that in the genic expression of the α -GPDH isozymes a single structural gene could be involved. Also, the origin of each molecular form could be a post-synthesis modification based on a polypeptide (Wright and Shaw, 1969; Bewley *et al.*, 1974; Bewley and Lucchesi, 1976; Bewley and Miller, 1979).

Our results permit two hypotheses concerning the genic expression of α -GPDH in *A. nuneztovari*: 1) The possible existence of two forms of the enzyme during development, which differ in intensity of coloration and, possibly, in electrophoretic mobility, which could confirm the independence of the two forms, indicating the existence of two loci. The lesser anodic form presents weak intensity and is characteristic of the larval stage, while the other, with a more anodic position, shows strong intensity and is characteristic of the pupal and adult stages. 2) The presence of a single locus and the possibility that the slight difference in mobility detected between the isozymes in the larval stage and those in the pupal and adult stages is a consequence of post-synthesis modifications. This has been considered by the above-mentioned authors Wright and Shaw, Bewley *et al.* and Bewley and Lucchesi, and amply analyzed in *D. melanogaster* by Bewley and Miller (1979).

Bewley and Miller (1979) also report a single form of the enzyme for larval and pupal stages and the appearance of two more isozymes in the late pupal stage and the

imago. After studies which involved thermal stability, kinetic parameters, different optimum pH's, isoelectric point (pI), electrophoretic mobility and other parameters, Bewley and Miller (1979) concluded that these isozymes observed in *D. melanogaster* are products of a single structural gene and that each isozyme shows a dimeric structure based on genetic and protein structural data. Considering these data, it is reasonable to assume that for *A. nuneztovari* a single locus is also involved and that the differences detected in electrophoretic mobility are a product of post synthesis modifications.

The importance of α -GPDH in the flight of insects led to the investigation of this system in various populations. The data suggest that the locus of α -GPDH varies little (Johnson, 1974; Powell, 1975; Machado, 1986). In the family Drosophilidae, in which α -GPDH was studied in 175 species, a low evolutionary rate was verified when compared to other enzymes analyzed. This fact was interpreted in terms of biochemical and physiological restrictions operating on the enzymes (Lakovaara *et al.*, 1977). These authors also reported that the small number of heterozygotes denotes the characteristic that the majority of mutants for the locus α -GPDH are harmful. These proposals are based on the premise that in insects α -GPDH has a relevant metabolic role (Lakovaara *et al.*, 1977). In other animals, in which α -GPDH is not involved in the production of energy, the enzyme is more polymorphic (Johnson, 1974; Powell, 1975; Lakovaara *et al.*, 1977).

Gillespie and Kojima (1968) and Kojima *et al.* (1970) suggest that the degree of polymorphism of the enzymes may be correlated with the physiological functions of the enzymes. The former also proposed that the enzymes that metabolize glucose are, in general, less variable than those of other systems. Although α -GPDH has been shown to be relatively invariable in different groups of insects, allelic variants of this enzyme were described for *D. melanogaster* (Grell, 1967; O'Brien and MacIntyre, 1972b; Miller *et al.*, 1975; Bewley and Miller, 1979; Gibson *et al.*, 1986), *C. pipiens* (Pasteur and Stordeur, 1976), the *A. gambiae* complex (Miles, 1978), *A. albopictus* (Tadano, 1984) and for bees (Machado, 1986). However, these examples are rare when compared to other enzymatic polymorphisms found in insects (Zera, 1981).

The results of the present study are in accordance with this trend, in that little variation was found for *A. nuneztovari*. The α -GPDH S and α -GPDH FS phenotypes were found indicating that the α -GPDH enzyme can be controlled by an α -GPDH locus with two codominant alleles, α -GPDH*F and α -GPDH*S. The α -GPDH*S allele was detected at a frequency of 0.970, and the α -GPDH*F allele was detected at low frequencies (0.030) in the population. Considering the criteria for 0.95 and 0.99 polymorphism proposed by Ayala *et al.* (1972), the α -GPDH locus is polymorphic only for the second criterion, i.e., when the frequency of the most common allele is not higher than 1%.

On the other hand, Zera (1981) has reported high levels of variability for α -GPDH in several Gerridae species (Hemiptera: Gerridae). He proposes that these high

levels of variability can be the result of the reduction of selection pressure on the locus resulting in the diminishing importance of the enzyme in these groups. Similar conclusions were made by Collier and MacIntyre (1977) for the genus *Drosophila* (Diptera: Drosophilidae). They also suggest that the increase in variability could be a consequence of the low degree of importance that this enzyme has for flight.

Thus, the low variability found in *A. nuneztovari* could be correlated to the importance that this enzyme has for the flight mechanism of this species. Agreeing with this proposal are the data on species of Gerridae, *Drosophila* and *P. claripalpis*. Also, in *Drosophila*, mutants deficient in the synthesis of α -GPDH, which exhibit absence of activity of this enzyme, are incapable of flight (O'Brien and MacIntyre, 1972b).

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RESUMO

A alfa-glicerofosfato desidrogenase mostrou, no desenvolvimento ontogenético de *A. nuneztovari*, um perfil eletroforético em que foi possível diagnosticar uma região de atividade, próxima a origem, que aumenta a intensidade à medida que se aproxima o estágio adulto. Desde o 1º estágio de larva até pupa com 4 horas, constata-se baixa atividade da enzima, mostrando atividade intensa a partir de pupas com 12 horas, até o adulto recém-emergido. A banda do estágio de larva mostrou uma pequena diferença de migração em relação à banda dos estágios de pupa e adulto. Este fato possibilitou aventar a hipótese da existência de duas formas da enzima, como produto de locos independentes ou, como é mais provável, e intensivamente demonstrado no gênero *Drosophila*, as duas serem produtos de um único loco, resultantes de modificações pós-síntese, a partir de um polipeptídeo. A constatação da atividade mais intensa no estágio adulto permitiu admitir que a enzima poderia apresentar concentrações maiores no tórax, exercendo funções relacionadas à atividade de voo da espécie. Também foi correlacionado com atividade de voo, o nível baixo de variação alélica observado.

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