In vitro Secretion of Four Dehydrogenases (ADH, MDH, IDH and α -GPDH) by Fat Bodies from Various Developmental Stages of the Mediterranean Fruit Fly, *Ceratitis capitata* (Diptera: Tephritidae)

Panagiotis George KATSORIS and Vassilis John MARMARAS¹

Department of Biology, University of Patras, Patras, Greece

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The developmental profile of four dehydrogenases of the fat body of *Ceratitis capitata* was found to be similar to that of the whole homogenate. Electrophoretic analysis revealed that the isozymic pattern of whole animal homogenate resembles that of the fat body, excluding MDH. Organ culture experiments demonstrated that the fat body secretes the four dehydrogenases into the incubation medium differentially. Moreover, electrophoretic analysis indicated that the secretion of fat body is specific, since one of the MDH isozymes was not secreted. Heat shock treatment resulted in the suppression of ADH activity to about 50% during the transformation of larvae to pupae. Moreover, it seemed to promote the secretive action of the fat body's isozymes.

Key words: dehydrogenase, *Ceratitis capitata*, fat body

INTRODUCTION

The insect fat body is a multifunctional organ, which combines many of the roles of the liver and adipose tissue of vertebrates. It is a site of protein synthesis and storage of materials for later use (PRICE, 1972, 1973). Furthermore, the fat body is responsible for intermediary metabolism (CHEN, 1972).

A number of proteins synthesized by the fat body have been demonstrated to be secreted into the haemolymph. The most striking examples are the larval serum proteins (CHRYSANTHIS et al., 1981) and the vitellogenins (WYATT and PAN, 1978; ENGELMANN, 1979; KUNKEL and NORDIN, 1985).

In the Mediterranean fruit fly *Ceratitis capitata*, the developmental changes, biosynthesis, secretion and uptake of larval serum proteins by the fat body have already been studied in some detail (CHRYSANTHIS et al., 1981; MINTZAS et al., 1983). The developmentally programmed synthesis, secretion and uptake of larval serum proteins demonstrate impressive regulation of the fat body's gene activity. Moreover, the temporally programming secretive and reabsorptive action of this tissue denotes a very close cooperativity between fat body and haemolymph. Furthermore, in the enzymic level we have demonstrated a differential secretion of the fat body peptidases during development (KATSORIS et al., 1981).

¹ To whom correspondence should be addressed.

In this paper we extend our study on the relationship of the fat body and haemolymph by studying the following four dehydrogenases: Alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH) and α -glycerophosphate dehydrogenase (α -GPDH). We have chosen these enzymes because the fat body is responsible for intermediary metabolism, and the four dehydrogenases are involved in the citric acid cycle, glycolysis and lipolysis. More specifically, we studied the four dehydrogenases regarding their qualitative and quantitative developmental changes in the fat body and their secretion. In addition, we tested the action of an environmental factor, the heat shock, that is known to differentiate the transcriptional program of cells (see review of ASHBURNER and BONNER, 1979).

MATERIALS AND METHODS

Animals. A laboratory stock of *Ceratitis capitata* was maintained at 25°C and 75% RH. Flies were fed on a 50% yeast- 50% sucrose medium and allowed to lay their eggs for 15 min. In order to ensure maximal synchronization, 25 mg of eggs was placed on plastic trays containing 150 g of larval food (500 ml H₂O, 30 g sucrose, 30 g yeast, 30 g toilet paper, 10 ml 1.5 N HCl, 10 ml 5% cholesterol in 25% ethanol and 8.5 ml 10% sodium benzoate in 60% ethanol). Under these conditions, embryogenesis lasts two d, larval stage six d and pupal stage about 10 d.

Preparation of crude extracts. Thirty animals of each developmental stage were homogenized with a glass potter homogenizer in 1 ml final volume of RINGER solution, containing 1 mM phenylthiouria (PTU). The homogenates were centrifuged at 10,000 g for 10 min and the clear supernatants were immediately used as an enzyme source.

Collection of fat body. Twenty animals from each of the selected developmental stages were dissected for each experiment in Ringer solution (128 mM NaCl, 1.8 mM CaCl₂, 1.3 mM KCl and 2.3 mM NaHCO₃) at 25°C. The fat bodies were rinsed three times after dissection and used for organ culture experiments or for homogenization (see below).

Organ culture. Freshly isolated fat bodies from 20 animals were incubated in 200 μ l Grace tissue culture medium (GIBCO), in airtight watch glasses with solid square bases for 2 h on a rotary shaker at 25°C. Then, the incubation medium was collected, centrifuged at 10,000 g for 10 min and used immediately or stored at -30°C. The tissue was rinsed three times and homogenized in RINGER solution to a final volume of 200 μ l. The homogenate was centrifuged at 10,000 g for 10 min and the clear supernatant was used directly or stored at -30°C.

Heat-shock treatment. The heat shock was performed in vials without food, each one containing 50 animals. Cotton plugs were moistened and forced into a position below the surface of a water bath into which the vials were immersed. The animals were pre-heated at 35°C for 30 min and then shocked at 45°C for 40 min. The survival rate of this treatment was about 94% (STEPHANOU et al., 1983). The animals were then dissected and their fat bodies used for organ culture experiments as previously described.

Enzyme assays. Enzymes were assayed by monitoring the increase of NADH or NADPH at 340 nm in a Gilford 250 S recording spectrophotometer equipped with a constant temperature circulating water pump, adjusted to 25°C. The assay methods used were based on those of DAY et al. (1974) for alcohol dehydrogenase (ADH), ALAHOTIS and BERGER (1978) for malate dehydrogenase (MDH), MILLER et al. (1975)

for α -glycerophosphate dehydrogenase (α -GPDH) and Fox (1971) for isocitrate dehydrogenase (IDH), with modifications for our animals. Varying the pH value of the buffers and the concentrations of coenzymes and substrates, we found that the optimal conditions for spectrophotometric assays were as follows:

ADH : 0.336% ethanol, 0.55 mM NAD in a 0.15 M phosphate buffer pH 8.2.

α -GPDH: 3.5 mM α -glycerophosphate, 3.5 mM NAD in a 0.1 M glycine-NaOH buffer pH 10.2.

MDH : 7.5 mM L-malic acid, 5 mM NAD in a 0.1 M glycine-NaOH buffer pH 10.2.

IDH : 0.2 mM isocitric acid, 0.1 mM NADP 42 mM MgSO_4 in a 0.1 M tris-HCl buffer pH 8.

Enzyme concentrations used were tested for linear relation to the resulting activities and the results are expressed in units per animal's fat body or units per animal (1U=1 mM S/min).

Starch gel electrophoresis. Starch gel electrophoresis was carried out using Tris-citrate buffer pH 7 (SHAW and PRASAD, 1970) at 4°C, for about 2 h at 80 mA constant current. The staining of the gels was performed according to ALAHIOTIS and BERGER (1978).

Protein determination. Protein was determined according to the method of LOWRY et al. (1951), with bovine serum albumin as a standard.

Fig. 1. Developmental profiles of the enzymic activity of four dehydrogenases from fat bodies. Values given are means of triplicated experiments $\pm$ SE and activity is expressed as Units/F.B. (i.e. fat body from one whole larva, pupa or adult). L: larva, WP: white pupa, P: pupa, A: adult. Numbers indicate days.

RESULTS AND DISCUSSION

To study the activities of the four dehydrogenases and their secretion from fat bodies during development, fat bodies from 20 animals were either homogenized and the homogenates were directly assayed or incubated in 200 μ l Grace complete medium for 2 h. After completion of incubation, samples from tissue homogenates and incubation media were assayed for enzymic activity as described in Materials and Methods.

The results are expressed in units of enzymic activity per fat body or per 10 μ l of incubation medium, reflecting the secretion of one animal's fat body during incubation. We avoided expressing the results in units of enzymic activity per mg of protein, since fat body differentially secretes and reabsorbs large quantities of protein such as larval serums. This differentiation depends on developmental stage (CHRYSAETHIS *et al.*, 1981; MINTZAS *et al.*, 1983; KALIAFAS *et al.*, 1984), so the relationship of enzymic

Fig. 2. Fat body's secretory disposition during development, expressed as the ratio of enzymic activity secreted versus the one remaining in tissue after the (a) organ culture experiment and (b) developmental profiles of the enzymic activity of four dehydrogenases remaining in the fat bodies (F.B.) or secreted in incubation media (I.M.), measured after 2 h organ culture in Grace complete medium. Values given are means from triplicated experiments $\pm$ SE and activity is expressed as Units/F.B. (i.e. fat body from one whole larva, pupa or adult) or units of enzymic activity secreted from one animal's F.B. after the completion of the organ culture. L: larva, WP: white pupa, P: pupa, A: adult. Numbers indicate days.

Fig. 2 (b)

activity to protein concentration is not useful for such developmental studies.

The enzymic activities of the fat body homogenates from various developmental stages are presented in Fig. 1. Three of them, namely the ADH, MDH and α -GPDH, showed a progressive increase of enzymic activity from early 3rd instar larvae to white pupae. On the other hand, the activity of these enzymes during the last pupal stage, where the fat body could be isolated, is very low. The activity of IDH remains rather constant during the larval and pupal stages tested.

Comparing these results with those obtained from total homogenates (data not shown), indicate that the developmental pattern of activity for at least ADH, MDH and α -GPDH are very similar in the fat body and in whole animal homogenates.

Figure 2a shows the ratio of enzymic activity secreted versus that remaining in the fat body after 2 h incubation in Grace complete medium for the four dehydrogenases assayed during development. Furthermore, Fig. 2b shows the actual activities of four

Fig. 3. Electropherograms from analysis of fat bodies and the corresponding media after organ culture experiments and during development (see Materials and Methods). Samples of duplicated experiments were run in parallel. L: larva, WP: white pupa, P: pupa, A: adult, IM: incubation medium, FB: fat body. Numbers indicate days.

dehydrogenases remaining in fat body and transferring into the medium. A common characteristic observed was that all enzymes tested were secreted from the fat body, but not to the same extent. In addition, the maximum enzyme secretive disposition for all four enzymes was observed in P8 age (Fig. 2a). However, the actual activities of four dehydrogenases secreted is almost the same to that of L6, where the secretive disposition is very low.

The activity of the four fat body dehydrogenases and their secretion into the incubation medium raises the following questions: (a) are the enzymic activities observed during development due to the same or different isozymes? and (b) if not all of the isozymes are secreted, which ones are?

In order to answer these questions, organ culture experiments and subsequent electrophoretic analysis were performed. Figure 3 shows that fat body secretes all isozymes into the incubation medium, with only one exception, an MDH isozyme migrating to the positive pole. This indicates specificity in isozymic secretion.

The above results demonstrated that fat body contains appreciable quantities of those enzymes and secretes them into the incubation medium. We can speculate that fat body may be a site of biosynthesis and secretion of these enzymes into the haemolymph.

Fig. 4. Fat body's secretive disposition during development, expressed as the ratio of enzymic activity secreted versus the one remaining in tissue after organ culture experiment. (○): values from control experiment; (●): values from previously heat-shocked animals (see Materials and Methods). Values given are means from triplicated experiments. L: larva, WP: white pupa, P: pupa, A: adult. Numbers indicate days.

Fig. 5. Developmental profiles of ADH activity of fat body. (○): values from control experiment; (●): values from previously heat-shocked animals (see Materials and Methods). Values given are means of triplicated experiments $\pm$ SE and activity is expressed as Units/fat body. L: larva, WP: white pupa, P: pupa, A: adult. Numbers indicate days.

To elucidate whether the secretive activity of fat body was changed according to the environmental conditions, we shocked animals of different developmental stages (see MATERIALS AND METHODS) and their fat bodies were isolated and incubated as previously described, as were isolated fat bodies from untreated animals. Measuring the secretive disposition of fat bodies from heat-shocked animals (ratio of enzymic activity secreted versus that remaining in tissue in organ culture experiment) and comparing it with that obtained from untreated animals (Fig. 2) suggests that heat-shock treatment promotes fat body secretive action throughout development and for all enzymes tested (Fig. 4). Regarding the isozymic pattern, no differences were observed between control and heat-shock treated animals (data not shown). However, the most interesting result of such treatment was the suppression of ADH activity, which reached about one half of that of the control during the white pupal stage and one-day old pupal stage (Fig. 5). For the other three enzymes, the activities in the fat bodies of treated and control animals were similar.

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