Miscellanea : Lipase and unspecific esterase activity in the fat body of "Aedes aegypti" L.

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Objekttyp: **Article**

Zeitschrift: **Acta Tropica**

Band (Jahr): **32 (1975)**

Heft 3

PDF erstellt am: **09.08.2024**

Persistenter Link: https://doi.org/10.5169/seals-312092

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Lipase and Unspecific Esterase Activity in the Fat Body of *Aedes aegypti* L.

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Abstract

In the fat body of *Aedes aegypti* a very high unspecific esterase activity and a low lipolytic activity was found. The electrophoretic isozyme patterns of the unspecific esterases show only few changes in the different physiological stages. The activity of the unspecific esterases as well as of the lipase is especially high in young sugar fed and in blood fed mosquitoes which points to special energy requirements in these stages. The role of the unspecific esterases is discussed.

Introduction

The present study was undertaken in continuation of other investigations on the esterase patterns in organs of *Aedes aegypti* (BRIEGEL & FREYVOGEL 1973, GEERING & FREYVOGEL 1974, GEERING & OBERLIN 1975). The fat body as the main site of triglyceride storage in the mosquito female (VAN HANDEL 1965) is of special interest for esterase analysis. Nothing is known about the utilization of the large fat deposits laid down after a sugar or a blood meal except that they are needed by starving and hibernating females (CLEMENTS 1963). In contrast to other insects, in mosquitoes energy consuming processes such as flight are not sustained by lipids, but exclusively by glycogen (CLEMENTS 1955). By studying the changes in the esterase patterns during different physiological stages of the fat body it was hoped to obtain more information on lipid metabolism in this organ.

Unspecific carboxylesterases are thought to play an important role in fat mobilization (SUDDERUDDIN & TAN 1973) but it is not known if they are able to carry out this task alone or only in the presence of a specific triglyceride lipase. Thus, an attempt was made to identify an extra-digestive lipase activity in the fat body.

Material and methods

Females of *Aedes aegypti,* strain Segemaganga (BRIEGEL & KAISER 1973) were used in this study. Homogenate preparation and disc electrophoresis were performed as described by BRIEGEL (1972) and by BRIEGEL and FREYVOGEL (1973). Unspecific esterase activity was detected using α-naphtyl acetate as substrate and Fast Blue salt RR as coupling dye. Acetylcholinesterases were identified by the method described by GEERING & FREYVOGEL (1974). The nomenclature of the bands was the same as used in other publications (BRIEGEL & FREYVOGEL 1973, GEERING 1973, GEERING & FREYVOGEL 1974). The total esterase activity (e/♀) is expressed as the integrated area of the gel records registered by a photometer.

The esterase activity was checked in different stages of sugar fed mosquitoes (0–50 days after emergence) as well as of mosquitoes receiving a blood meal 3 days after emergence (Fig. 1). Lipase activity was determined by using the commercially prepared coconut oil emulsion, Lipostrate CB (Calbiochem) as substrate. The assay system contained 1.15 ml Tris-maleate buffer pH 8, 50 mg bovine

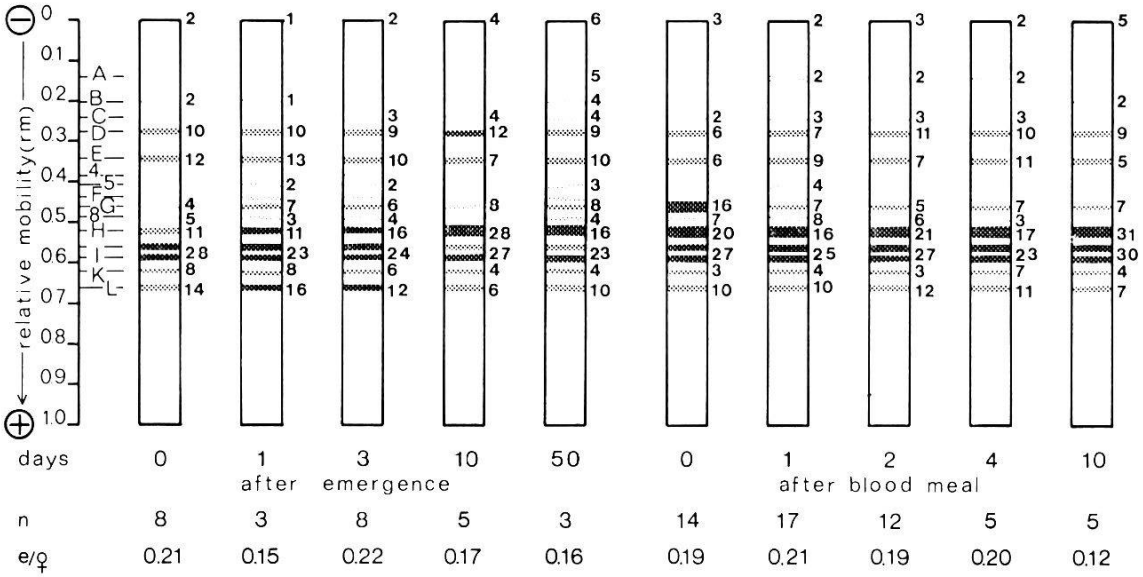

Fig. 1. Esterase patterns of the fat body in different physiological stages. Numbers to the right of the gel strips = relative activity of the bands (percentage of total activity); n = number of experiments; e/♀ = total esterase activity per female.

serum albumin, Fraction V (Sigma), 0.05 ml Lipostrate and 0.1 ml homogenate containing 20 fat bodies. Lipase activity is expressed as mμ moles of free fatty acids released by 1 fat body in 30′ at 37 °C. The free fatty acids were determined by the method of LAUWERYS (1969) with 1,5-diphenylcarbohydrazide as complexing agent (MAHADEVAN et al. 1969).

Results and Discussion

The fat body of *Aedes aegypti* L. produces the same esterase isozymes as other organs (BRIEGEL & FREYVOGEL 1973, GEERING & FREYVOGEL 1974); however, quantitatively they occur in a specific distribution (Fig. 1). No marked changes can be observed in the esterase pattern during the life time of the mosquito except from some quantitative alterations of a few bands. Through all stages, the predominant bands are D, E, L and especially H and I. A similar distribution of the fractions is found in the ovary of the mosquito (GEERING & OBERLIN 1975). Bands A, B and C, identified as acetylcholinesterases, are poorly represented in this organ. Fractions, which undergo quantitative changes, are band H, which increases from the day of emergence until the age of 10 days, and one of the fractions of the double band I, which decreases in sugar fed but not in blood fed mosquitoes after ten days. Band G greatly increases immediately after a blood meal. This phenomenon was also observed in the midgut epithelium and is discussed elsewhere (GEERING & FREYVOGEL 1974). The total esterase activity per female is very high in the fat body compared to other organs studied (BRIEGEL & FREYVOGEL 1973, GEERING & FREYVOGEL 1974). Since the fat body is the main site of triglyceride storage (VAN HANDEL 1965), this increased esterase acivity supports the hypothesis of the involvement of unspecific esterases in lipid metabolism (SUDDERUDDIN & TAN 1973). It would then reflect a high and constant turnover of metabolites for energy production in optimally fed mosquitoes. The total esterase activity per female does not show great variations during the different physiological stages. Slightly increased values can be observed in fat bodies just after emergence (Fig. 1, day 0), in 3-day-old sugar fed and in blood digesting females. The increased occurrence is not easy to explain; it might be

Table 1. Lipase activity of the fat body in different physiological stages

Days after emergence (sugar fed)	mμ moles FFA/fat body/30 min. (n=3–5)
0	14.3
3	9.1
7	7.8
Days after blood meal	
1	13.6
3	1.9
7	9.1

an expression of a well developed fat body or of special energy requirements in these stages.

In order to find out if a specific lipase is represented among the separated esterases, gels were sliced and after extraction each part checked for lipase actitvity. Triglyceride splitting activity could only be identified in the starting fraction. This shows that a specific lipase exists but that none of the so-called unspecific esterases is able to split triglycerides. OKUDA and FUJII (1968) suggest that liver lipase is a complex of liver esterase and lipid. This could be an explanation for the impossibility of separation in the gel system described. Further experiments were carried out on crude homogenates of the fat body. Lipase activity is very low in the fat body compared to the unspecific esterase activity (Tab. 1). This is in agreement with the findings of GILBERT et al. (1965). It supports the statement that lipases alone could not cope with all energy demands and that unspecific carboxylesterases might play an important role in fat mobilization (SUDDERUDDIN & TAN 1973). Lipase activity, as well as unspecific esterase activity, is relatively high in 0 day old mosquitoes. On the first day after emergence, increased fat mobilization for special energy requirements such as completion of morphogenesis could be assumed (HECKER et al. 1974). The increased lipase activity after a blood meal could be in connection with egg development. DUTKOWSKI and ZIAJAKA (1972) suggested that the fat body lipids may be an extra-ovarian source of yolk lipids. In mosquitoes it was shown that triglyceride utilization is independent of the endocrine system (VAN HANDEL & LEA 1970) but an indirect influence by the developing ovary may not be excluded.

Acknowledgements

The author wishes to thank Prof. T. A. Freyvogel for his valuable comments on this work. This work was supported by the "Schweiz. Nationalfonds zur Förderung der wissenschaftlichen Forschung", grant No. 3.7630.72.

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