STUDIES ON TRIACYLGLYCEROL ESTER HYDROLASE ACTIVITY IN MUSCLE DURING LARVAL DEVELOPMENT OF PAPILIO DEMOLEUS (LINNAEUS)

R. D. Bodare and Manisha R. Gejage

KEYWORDS

Triacylglycerol ester hydrolase
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Studies on triacylglycerol ester hydrolase activity in muscle during larval development of citrus swallowtail, *Papilio demoleus* (Linnaeus) have been studied. The larval muscle triacylglycerol ester hydrolase revealed optimum pH 7.6, incubation time 25 minutes, temperature 37ºC, enzyme concentration 1%, substrate concentration 6% and Michaelis-Menten constant $0.192 \times 10^{-2}$ mM. The triacylglycerol ester hydrolase mainly hydrolysed the triglycerides to diglycerides and fatty acids. The gradual increase in muscle triacylglycerol ester hydrolase activity was observed from 6-day larvae to 9-day larvae and decrease from 9-day larvae to 14-day larvae. The maximum lipase activity was observed in 9-day larvae. The physiological role of triacylglycerol ester hydrolase in muscle during larval development of *P. orbonalis* (Guenee) has been reported in present paper.

**ABSTRACT**

The *P. demoleus* (Linnaeus) causes damage to citrus plants in Africa, Taiwan, India and Japan. The young caterpillar is brown with white markings and colour changes to green with brown bands across the back when full grown. It initially feeds on small leaves attacking larger ones as it grows older. It feeds for about 14 days in summer. Lipids are essential structural components of the cell membrane and cuticle, they provide rich source of metabolic energy for periods of sustained energy demand, they facilitate water conservation both by the formation of an impermeable cuticular barrier and by yielding metabolic water upon oxidation and they include important hormones and pheromones (Pol and Gejage, 2002). A few studies have been carried out in *P. demoleus* (Linnaeus) which is pest of citrus (Badawi, 1981; Radke and Kandalkar, 1988; Bhan and Singh, 1997; Moonen, 1991; 1999; Bhan and Singh, 1997; Matsumoto, 2002; Pathak and Rizvi, 2003; Chen et al., 2004; Guerrero et al., 2004; Homziak and Homziak, 2006; Pandey et al., 2012).

Some attempts have been made to investigate triacylglycerol ester hydrolase activity in insect species (Arreguin et al., 2000; Pol and Sakate, 2000; Pol and Gejage, 2002; Pol and Salunkhe, 2001a;b; Ponnuvel et al., 2003; Rosetto et al., 2003; Arrese et al., 2006; Patel et al., 2005; Grillo et al., 2007; Orscal et al., 2007; Mradakovic et al., 2008; Zibaee et al., 2008; Bickel et al., 2009; Gejage and Awate, 2009; Horne et al., 2009; Olofsson et al., 2009; Arrese and Soulages, 2010; Gejage and Gejage, 2010a, b, c; Ademolu, 2011, Christeller et al., 2009, 2011; Chattopadhyay, 2011; Khosravi et al., 2011; Markwick et al., 2011; Schafer et al., 2011; Tierno de Figueroa et al., 2011). The information on triacylglycerol ester hydrolase in muscle during larval development of *P. demoleus* (Linnaeus) is rather scanty.

In the present investigation, an attempt has been taken to evaluate triacylglycerol ester hydrolase activity in muscle during larval development of *P. demoleus* (Linnaeus) which is mainly concerned with release of energy for their active life and structural components of larval growth.

**MATERIALS AND METHODS**

The culture of *P. demoleus* (Linnaeus) was maintained in the laboratory on the natural food of citrus leaves. The larval developmental stages from 6-day to 14-day larvae were taken for study of triacylglycerol ester hydrolase activity. For the enzyme preparation larval muscles were isolated under ice cold distilled water, weighed and homogenized in the cold distilled water using a ground glass mortar and pestle. The homogenate were diluted with cold distilled water so as to get 1% (wt/vol) concentration. Such homogenate were used for the assay of triacylglycerol ester hydrolase activity. The triacylglycerol ester hydrolase was assayed by the method of Hayase and Tapple (1970). The assay system contained 0.25 mL of 6 % olive oil dispersed in gum acacia; 1.0mL of 0.2 M tris-maleate buffer pH 7.6 and 0.25mL of 1 % (wt/vol) enzyme solution in a total volume of 1.5mL. The incuba-
tions were carried out in a shaker with a continuous shaking for 25 minutes in glass stoppered vessels at 37°C. The reaction was stopped with 2mL of Cu-TEA reagent (1N acetic acid: 1M 2, 2', 2" trinitrilloethanol:6.45% Cu(NO₃)₁₋(1:9:10,v/v/v)). The colour was developed by the addition of 1mL of 0.5% solution of mixture of diphenyl carbazide and diphenylcarbazid (5:95 w/w) in methanol. At the end of incubation period, the liberated fatty acids were measured colorimetrically according to Itaya (1977).

RESULTS

Larval developmental period of *P. demoleus* (Linnaeus) is of 14-days. Triacylglycerol ester hydrolyase activity in muscle during larval development of *P. demoleus* (Linnaeus) is shown in Fig. 1. The larval muscle triacylglycerol ester hydrolyase revealed optimum pH 7.6, incubation time 25 minutes, temperature 37°C, enzyme concentration 1%, substrate concentration 6% and Michaelis-Menten constant 0.192×10⁻² mM. The triacylglycerol ester hydrolyase mainly hydrolysed the triglycerides to diglycerides and fatty acids. The gradual increase in triacylglycerol ester hydrolyase activity was observed from 6-day larvae to 9-day larvae and decrease from 9-day larvae to 14-day larvae. The maximum triacylglycerol ester hydrolyase activity was observed in 9-day larvae.

DISCUSSION

Muscles from *Hyalophora cecropia*, *Schistocerca gregaria* and a number of other species have lipases which act more rapidly on diacylglycerols and monoacylglycerols than on triacylglycerols (Crabtree and Newsholme, 1972). Lipid is stored in the fat body in the form of triglyceride and transported to the muscles by the haemolymph in the form of diglyceride or free fatty acids and then the fat body must contain lipase activity (Candy and Kilby, 1975). In the muscle, a strong correlation was found between the activities of lipases and the known use of lipid as a fuel in *Periplaneta americana*, *Locusta migratoria* and *Pola* *adjuncta*. Lipase activity was lowest in the cockroach (carbohydrate based flight metabolism) intermediate in the *Locust* (both carbohydrate and lipid fueled flight) and highest in the moth, *P. adjuncta* (a non-feeding, lipid catabolizing adult) flight muscle (Male and Storey, 1981).

Most holometabolous insects accumulate large quantities of fat during their larval life. In some insects lipid synthesis increases sharply in the middle of the Vth instar, hence in the early pupal stages relative lipid content is about two or three times that of the early stages of the Vth instar (Lawrence et al., 1986). The lipid content of the muscle was 1.96 and 12.96% on fresh and dry weight basis respectively in the larvae of *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae) (Uner, 1988). In Holometabola certain larval muscles were retained in the adult, other muscles were differentiated by nuclear division and disintegration of the myofibrillae, then strengthened and modified by myoblasts into new fibrillae and newer nuclear arrangements, some larval muscles were completely dissolved by phagocytosis or by extra-cellular digestion and reformed into adult muscles (Mani, 1994). In general larval muscles were histolyed and adult muscles rebuilt in the pupa. The alimentary canal was extensively remodeled at metamorphosis in species which have different larval and adult diets. The midgut was probably completely renewed in all holometabolous insects, usually being reformed from the regenerate cells at the base of the epithelium. Sometimes this process occurs twice, once on the formation of the pupa and again when the adult tissues are forming and it was suggested that these can be assimilated and used in the reconstruction (Yadav, 2004).

The level of lipase activity was maximal at pH 7 to 8 in muscle of 6 day larvae (wandering stage) after which it declined in 7 day larvae of blowfly, *Calliphora erythrocephala* (Price, 1975). The lipase activity was maximal at the broad pH 8.6, 1% enzyme, 10 minutes of incubation time and increase in activity from 3-day to 4-day larval muscle of *C. rufilacies* (Pol and Savant, 1995).

In the present work, partial characterization of larval muscle triacylglycerol ester hydrolyase of *P. demoleus* (Linnaeus) pH 7.6, incubation time 25 minutes, temperature 37°C, homogenate (enzyme) concentration 1%, substrate concentration 6% and Michaelis-Menten constant 0.192×10⁻² mM suggests that the larval muscle triacylglycerol ester hydrolyase activity was affected by pH and shows an optimal activity at a pH 7.6. The optimal pH generally reflects the pH of the environment in which the enzyme normally functions. Michaelis-Menten constant 0.192×10⁻² mM indicates high affinity of triacylglycerol ester hydrolyase to the olive oil emulsion. The findings noted herein indicates that extra digestive alkaline triacylglycerol ester hydrolyase (EC 3.1.1.3) exist in the larval muscle homogenate of *P. demoleus* (Linnaeus) and due to this triacylglycerol ester hydrolyase diglycerol may be released from the muscle and served as important intermediate in the breakdown of triacylglycerol. Similar lipolytic findings were noted by above workers.

In the present study, gradual increase in triacylglycerol ester hydrolyase activity from 6-day larval muscle to 9-day larval muscle of *P. demoleus* (Linnaeus) indicates energy utilization in a series of moultings and energy required for their active life may be supplied by triglyceride catabolism. In the present
work, decrease in enzyme activity from 9-day larval muscle to 14-day larval muscle suggests the decreased rate of hydrolysis of lipid and triglycerides may accumulate gradually and attains high concentration in prepupae.

In the present study, maximum triacylglycerol ester hydrolyase activity in 9-day larval muscle of P. demoleus (Linnaeus) indicates the possible mobilization of lipid in most active feeding larval stage which required more energy and structural components for larval growth. The minimum triacylglycerol ester hydrolyase activity of 6-day larval muscle as compared to 9-day larval muscle of P. demoleus (Linnaeus) suggests histogenesis and early developmental period of larval development. Similar findings were noted by above authors.

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