Odonatologica 21(1): 111-117 *March 1, 1992*

MIDGUT DIGESTIVE ENZYME ACTIVITY IN THE DRAGONFLY, *TRAMEA VIRGINIA* (RAMBUR) (ANISOPTERA: LIBELLULIDAE)

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Received June 12, 1990 / Revised and Accepted June 19, 1991

The optimal conditions for the activity of the principal midgut digestive enzymes, e.g., amylase, protease and lipase have been ascertained in the final instar larva and adult of *T. virginia*. Although the peak activity of the enzymes is at similar pH, buffer concentration and temperature, a wide discrepancy occurs among the other parameters, particularly the substrate and enzyme concentrations and the incubation period. Midgut digestive enzyme activity is higher in the final instar larva than in the adult.

INTRODUCTION

Although there have been extensive investigations on midgut digestive enzymes in various groups of insects, no substantial information is available on Odonata (DADD, 1970; HOUSE, 1974; APPLEBAUM, 1985).

The present work was, therefore, undertaken to determine the optimal conditions for activity of the major midgut digestive enzymes, i.e., amylase, protease and lipase, in the final instar larva and adult of *Tramea virginia*.

MATERIAL AND METHODS

The enzyme solution was prepared according to APPLEBAUM et al. (1964) with some modifications. The head and terminal part of the abdomen were chopped off and the entire digestive tract was pulled out and immediately transferred to ice cold insect Ringer's solution. The Malpighian tubules, adhering tissues and gut contents, along with the peritrophic membrane, were carefully removed and the midgut was then weighed to ± 1 mg. The midgut was thoroughly homogenized in icecold citrate phosphate buffer (pH 6.8) and made up to 1 ml. The homogenate was centrifuged for 15 min at about 2000 revs/min and the clear supernatant was stored under a drop of toluene at about 10° C until required.

Amylase activity was determined according to Noelting & Bernfeld's method, modified by ISHAAYA & SWIRSKI (1970), using 3,5-dinitrosalicylic acid reagent. Absorbancy of the sample was measured at 550 nm against a reagent blank in a spectrophotometer. The amylolytic activity was expressed as the weight of the reducing sugar (glucose) produced by the enzymatic action per unit weight of gut per unit time, using glucose as the standard.

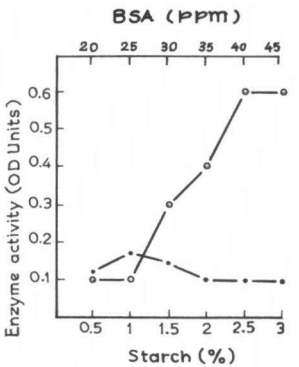

Fig. 1. Substrate concentration and enzyme activity. [o——o amylase, ●——● protease].

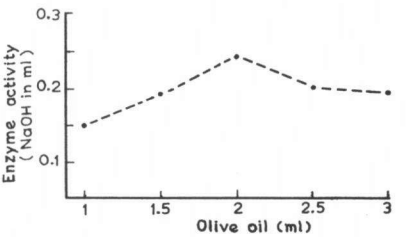

Fig. 2. Substrate concentration and lipase activity.

Protease activity was determined by the method of SNELL & SNELL (1971). The difference in absorbance was measured at 660 nm. The method of LOWRY et al. (1951) was used for protein measurement. Lipase activity was determined by the method of CHERRY & CRANDALL (1932).

In all experiments, the value of enzyme activity of each assay is the mean of 4-5 replicates.

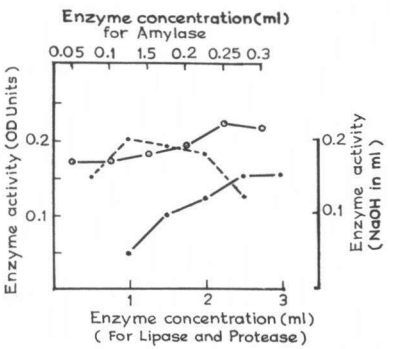

Fig. 3. Enzyme concentration and enzyme activity [o——o amylase, ●——● protease, ●----● lipase].

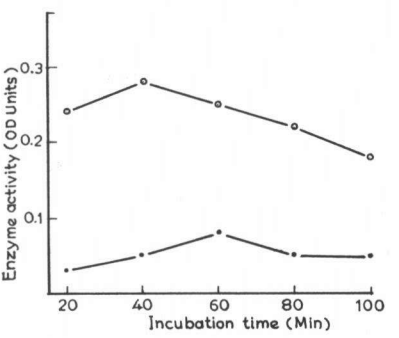

Fig. 4. Incubation time and enzyme activity [o——o amylase, ●——● protease].

RESULTS

The optimal physico-chemical factors i.e., substrate, hydrogen ion and enzyme concentration, temperature and incubation period promoting maximal midgut digestive enzyme activity in the final instar larva were determined separately. To determine the optimal condition of a particular parameter, a wide range of concentrations (substrate, pH, buffer and enzyme solutions) or linear propagations

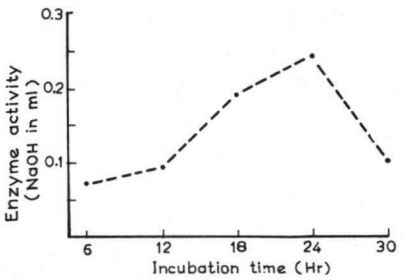

Fig. 5. Incubation time and lipase activity

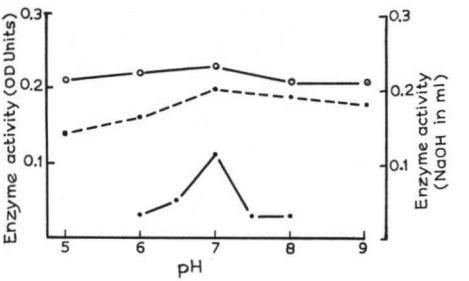

Fig. 6. Enzyme activity and pH [o——o amylase, ●——● protease, ●----● lipase].

(temperature and incubation period) of that factor were tested, while all the other factors were kept strictly constant at their functional range.

The midgut digestive enzymes show maximal activity at an optimal concentration of their respective substrate present in the incubation media. The present

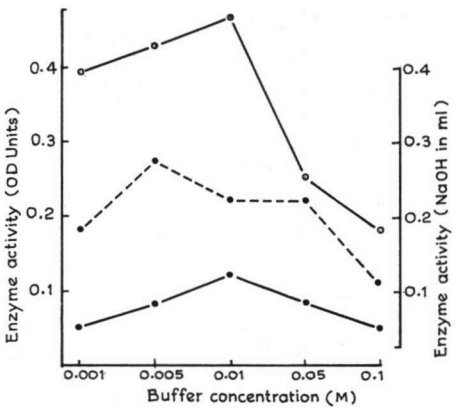

Fig. 7. Buffer concentration and enzyme activity [o——o amylase, ●——● protease, ●----● lipase].

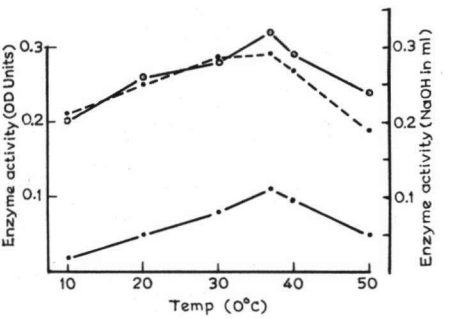

Fig. 8. Temperature and enzyme activity [o——o amylase, ●——● protease, ●----● lipase].

data reveal that amylase, protease and lipase activity attain the peak level at 2.5% starch, 25 ppm bovine serum albumen and 2 ml olive oil concentrations, respectively (Figs. 1 & 2). Similarly, the incubation media containing the enzyme concentration of 0.25, 2.5 and 2.0 ml provoke maximum activity of amylase, protease and lipase, respectively (Fig. 3). Complete digestion of the respective substrates by amylase, protease and lipase requires incubation periods of 40, 60 and 1440 min (24 h), respectively (Figs 4 & 5). The present experiments (Figs 6-8) unequivocally show that all these midgut enzymes in the final instar larva of *Tramea virginia* attain peak activity at one and the same hydrogen-ion concentration (pH 7), buffer concentration (0.01M) and temperature (37°C). The optimal physico--chemical conditions required for the midgut digestive enzyme activity are summarized in Table I.

Table I
Optical physico-chemical conditions required for the midgut digestive enzyme activity in *T. virginia*

Physico-chemical conditions	Midgut digestive enzymes		
	Amylase	Protease	Lipase
Substrate concentration	2.5% Starch	25 ppm BSA	2 ml Olive Oil
Hydrogen-ion conc. (pH)	7	7	7
Buffer conc. (M)	0.01	0.01	0.01
Enzyme conc. (ml)	0.25	2.5	1.0
Temperature (°C)	37	37	37
Incubation period (min)	40	60 (1 h)	1440 (24 h)

BSA: Bovine serum albumen. Units are given in parentheses. All values remained constant without any variation as found in 8-10 readings, while determining each optimal condition.

The midgut digestive enzymes in the adult dragonfly also exhibit peak activity at similar optimal conditions to those found in the larvae. Moreover, intensity of the amylase, protease and lipase activity in the larva is considerably higher than that in the adult (Tab II).

Table II
Optimal activity of the midgut digestive enzymes in *T. virginia*

Enzyme	Stage		Unit
	Larva	Adult	
Amylase	6.66 ± 0.24	4.44 ± 0.10	mg glucose liberated/mg midgut/ml enzyme solution/min.
Protease	0.112 ± 0.001	0.094 ± 0.001	mg protein liberated/mg midgut/ml enzyme solution/min.
Lipase	0.478 ± 0.006	0.465 ± 0.002	mg oleic acid liberated/mg midgut/ml enzyme solution/min.

Each value represents the mean of 8-10 readings. ± standard error (SE)

DISCUSSION

Histological and histochemical studies on the alimentary canal of the final instar larva and adult of *T. virginia* indicate that the midgut epithelium, and particularly the columnar cells exclusively, form a site of absorption and digestion of carbohydrates, proteins and lipids (MUTHAL, 1989) similar to that in *Aeshna cyanea* (ANDRIES, 1976) and other insects (TURUNEN, 1985).

In *T. virginia*, the substrate enzyme activity curve evokes a general feature that the enzyme activity remains almost constant after achieving the optimal level and is thus in agreement with the findings of earlier workers (HORI, 1970a, 1970b). Similarly, the maximum midgut amylase, protease and lipase activity in *T. virginia* occurred at pH 7, similar to that in the dragonfly, *Brachythemis contaminata* (BALASUBRAMANIAN & PALANICHAMY, 1985) and the Almond fruit borer (ISHAAYA & PLAUT, 1974). In insects, the optimal pH for amylase activity ranges from 5.5 to 9.5, and protease and lipase activity occurs mostly at neutral pH or on the alkaline side, but in the larva and adult of *T. virginia*, the maximal activity of all the midgut enzymes appeared at neutral pH, which seems to represent the species-specific characteristic feature (HOUSE, 1974).

It becomes obvious from the findings of earlier workers that the midgut pH in insects mostly falls in the range of 6-8 and alkaline pH is more usual in the phytophagous insects than in the carnivorous ones (DAY & WATERHOUSE, 1953; HOUSE, 1974). In the larva and adult of *T. virginia* not only was the pH almost neutral but all enzymes showed maximal activity at the optimal buffer concentration of the media, i.e., 0.01 M, signifying the well buffered midgut as an adaptation to a carnivorous mode of feeding.

In the larva and adult of *T. virginia* the rate of substrate hydrolysis increased with enzyme concentration to a maximum after which it remained constant or declined slowly, in a similar manner to that reported in other insects (HORIE, 1959; KHAN & FORD, 1962; SRIVASTAVA & AUCLAIR, 1962; YANG & DAVIES, 1968; HORI, 1970a, 1970b; AGRAWAL, 1976; SINHA, 1976).

During the present study amylase, protease and lipase activity increased with increase in temperature from 10 to 37°C, but decreased at higher temperatures, suggesting that all the midgut enzymes are equally adapted to 37°C.

In the larva and adult of *T. virginia* amylase, protease and lipase activity required an optimal incubation period of 40, 60 and 1440 min (24 hrs), respectively, supporting the consideration of some workers that each midgut enzyme is adapted to the incubation period independently (ISHAAYA & SWIRSKI, 1970; HORI, 1970b; MANDAL et al., 1981).

Although the midgut enzymes' activity is found higher in the larva than in the adult, the conditions under which amylase, protease and lipase showed maximal activity were almost identical in both the larva and adult, suggesting that these

enzymes seem to be physiologically identical in both stages as in the Almond wasp, *Eurytoma amygdali* (ISHAAYA & PLAUT, 1974).

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