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https://doi.org/10.5109/23886

出版情報：九州大学大学院農学研究院紀要. 34 (1/2), pp.37-42, 1989-11. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 34 (1•2), 37-42 (1989)

Carbohydrate Enzymes in House Fly *Musca domestica* Larvae : Biochemical Properties and Toxicological Significance

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(Received March 23, 1989)

The optimum temperatures and pHs for trehalase, amylase and invertase enzymes of *Musca* ***domestica*** were ***52, 37*** and 42°C, and 5.5, 6.5 and 6.0, respectively. The activity was linear up to 90 min and 60 min was chosen as the reaction time. K_m values for invertase, trehalase and amylase were 0.0185 M, 0.00676 M and 0.294%, respectively. *Musca* ***domestica*** larvae fed for two days on dietary concentrations of 5 and 10 ppm 2-methoxy-5-phenyl-1, 3, 2-oxazaphospholidine 2-sulfide (5-PMOS) resulted in weight gain inhibition of 39 and 70%, relative to control, correlating well with the suppression in the soluble gut trehalase activity. The digestive amylase and invertase enzymes were not affected by this compound. Hence, the trehalase system, which plays an important role in energy supply in insects, could be or closely relates to the primary site for the observed weight gain inhibition after 5-PMOS treatment. Similar results were observed with 4-isobutyl-2-methoxy-1, 3, 2-oxazaphospholidine 2-sulfide (iBMOS). This system is suggested as a biochemical parameter to assess retardation of growth caused by various insecticides.

INTRODUCTION

Enzyme systems affecting insect growth such as trehalase which in insects degrades trehalose for internal energy supply (Ishaaya, 1986 : Wyatt, 1967) and digestive enzymes such as protease, amylase and invertase (Ishaaya, 1986) have been used to assess retardation of larval growth caused by secondary compounds present in plants or by treatment of insecticides exhibiting antifeeding activity (Ascher and Ishaaya, 1973 : Ishaaya and Casida, 1975). This paper describes the optimization of the trehalase, amylase and invertase enzymes in *M. domestica* larvae along with their toxicological significance using potential insecticides, such as 4-isobutyl-2-methoxy-1, 3, 2-oxazaphospholidine 2-sulfide (iBMOS) and 2-methoxy-5-phenyl-1, 3, 2-oxazaphospholidine 2-sulfide (5-PMOS).

MATERIALS AND METHODS

Chemicals

4-Isobutyl-2-methoxy-1,3, 2-oxazaphospholidine 2-sulfide (iBMOS) was prepared in the presence of triethylamine from methoxy phosphorodichloridothionate and

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L-leucinol (Eto *et al.*, 1981). 2-Methoxy-5-phenyl-1, 3, 2-oxazaphospholidine Z-sulfide (5-PMOS) was similarly synthesized from 2-amino-1-phenylethanol (Wu ***et al.,*** 1988).

Rearing and bioassay

A house fly diet (10 g, Oriental Kobo, K. K., Tokyo, Japan) was mixed with 35 ml acetone solution containing the test compound, or with 35 ml acetone alone as a control. After the solution had been evaporated under a hood for 2 days at room temperature, 12 g of water was added to the diet and thoroughly mixed. The diet was distributed in 1.5 g portions of ten replicates in test tubes (13 x 150 mm). Three larvae of susceptible (SRS) house fly, l-2 days old weighing 2 ± 1 mg each, were introduced into each test tube along with a thin piece of cotton placed over the diet to enable pupation. The tubes were kept at 28°C until pupation and emergence were complete. For weight gain measurement and enzyme activity determination, 2 larvae weighing 12± 1 mg each were introduced into 5 replicates of 6 test tubes each along with 1.3 g portions of diet and the tubes were held at 28°C for 2 days. The weight gain along with the larval trehalase, amylase and invertase activities were determined. Assays run with solvent treatments containing no chemicals were used as control. The data for both biological and enzyme tests are reported as means with SE values.

Preparation of larval enzymes and enzyme assays

The guts of 15-25 *M. **domestica*** larvae (weighing 19±1 mg each) were dissected free from other tissues by cutting off the larval head and removing the alimentary canal onto a wet filter paper (Hirashima et ***al.,*** 1989a : Ishaaya and Casida, 1975) and then homogenized in cold distilled H_2O (lo-fold relative to larval weight), using a chilled glass-Teflon Potter-Elvehjem tissue grinder tube. The homogenate was centrifuged for 15 min at 10,000 g at 2°C, the supernatant fraction being used for the enzyme assays. The protein level (Lowry ***et al.,*** 1951) in the enzyme solution was 755f 12 μg for the amylase assay, and 378±6 μg for the trehalase and the invertase assays. Trehalase, amylase and invertase activities were assayed colorimetrically on the basis of liberated glucose as previously described (Hirashima et al., 1989a ; Ishaaya and Casida, 1975) under conditions found to be the optima for *M. **domestica*** larvae.

The reaction mixtures for amylase consisted of 0.2 ml of 4% (w/v) starch, 0.1 ml of 0.2 M phosphate buffer (pH 6.5) and 0.1 ml of enzyme solution. The optimum reaction mixture for trehalase and invertase consisted of 0.2 ml of 4% (w/v) trehalose (for trehalase) or 0.2 ml of 4% (w/v) sucrose (for invertase), 0.1 ml of 0.2 M phosphate buffer (pH 5.5 and 6.0 for trehalase and invertase, respectively) and 0.2 ml enzyme solution. After 60 min incubation at 37, 42 and 52°C for amylase, invertase and trehalase, respectively, 0.8 ml of 3, 5-dinitrosalicylic acid (DNSA) reagent (Noelting and Bernfeld, 1948) was added. The mixture was heated for 5 min at 100°C, and then immediately cooled in an ice-bath. The absorbancy at 550 nm was determined in E units with a Shimadzu UV-2100 spectrophotometer.

RESULTS AND DISCUSSIONS

Biochemical properties of M. *domestica* larval gut enzymes

The optimum conditions for enzyme assays were evaluated in a series of prelimi-

nary experiments, in which individual factors were varied, all others being kept at the optimum.

The optimum temperatures and pHs for trehalase, amylase and invertase were 52, 37 and 42°C (Fig. 1), and 5.5, 6.5 and 6.0 (Fig. 2), respectively. The activity was linear up to 90 min (Fig. 3) and 60 min was chosen as the reaction time for all the enzyme assays. K_m values for invertase, trehalase and amylase were 0.0185 M, 0.00676 M and 0.294%, respectively (Fig. 4). One E unit of the absorbancy at 550 nm was 0.60 mg of glucose for trehalase and invertase, and 0.45 mg of glucose for amylase, respectively (Fig. 5).

Toxicological significance of carbohydrate enzymes of *M. domestica*

At sublethal concentrations, the five-membered cyclic phosphorothionates (iBMOS and 5-PMOS) resulted in a strong retardation of larval growth of the house fly larvae.

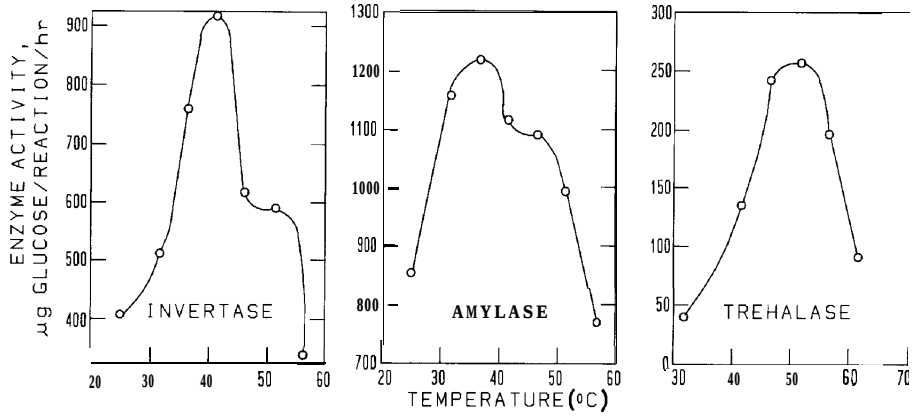

Fig. 1. Temperature optimum curves for trehalase, amylase and invertase of house fly ***M. domestica*** larvae.

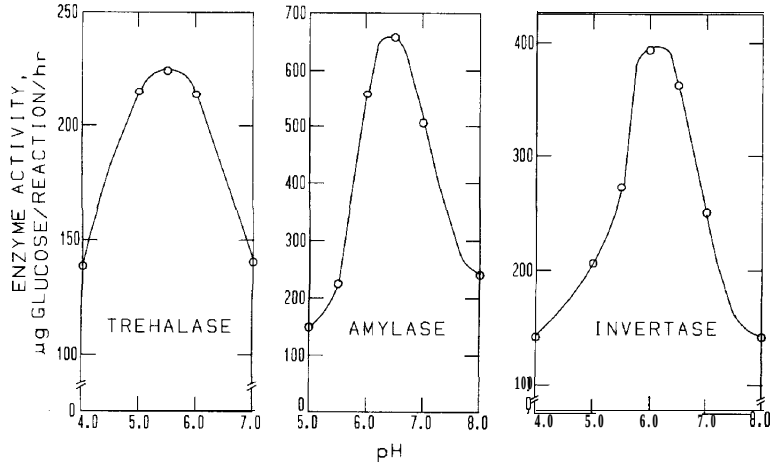

Fig. 2. pH Optimum curves for trehalase, amylase and invertase of house fly larvae.

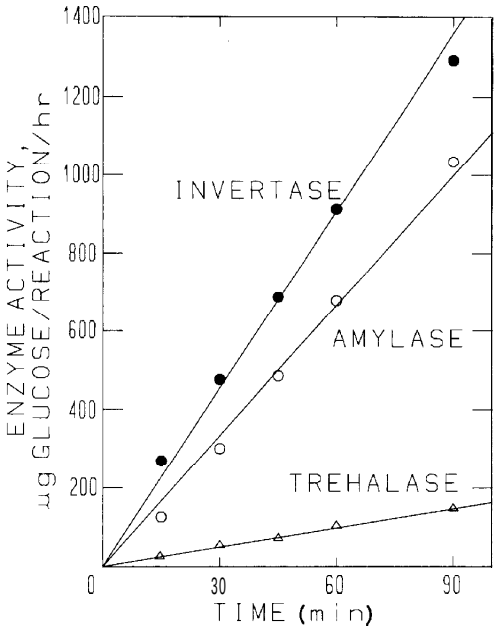

Fig. 3. Rate curves for trehalase, amylase and invertase of house fly larvae. Amylase activity was measured after dilution with water (1 : 1 volume) at the end of the reaction with DNSA reagent.

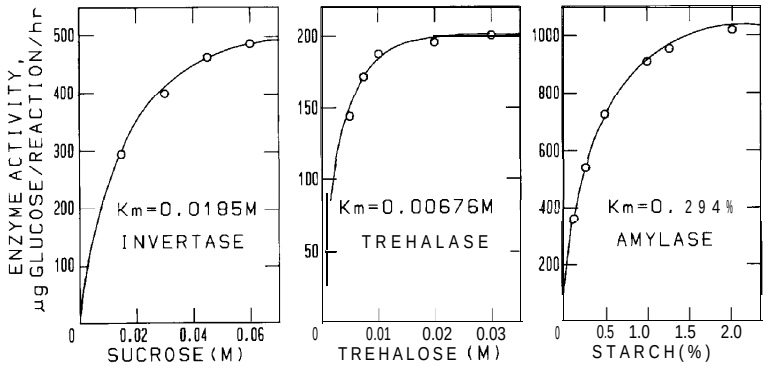

Fig. 4. Effect of substrate concentrations on trehalase, amylase and invertase of ***M. domestica*** larvae.

Hence, the digestive amylase and invertase activities which are important in food digestion and the trehalase enzyme in degradation of trehalose to glucose for energy supply (Ishaaya et al., **1986** : Wyatt, 1967) were assayed in larvae fed on diet containing these compounds.

House fly larvae fed for two days on dietary concentrations of 5 and 10 ppm 5-PMOS resulted in larval weight gain inhibition of 39 and 70%, correlating well with the suppression of the activity of the soluble larval gut trehalase (Table 1). 5-PMOS

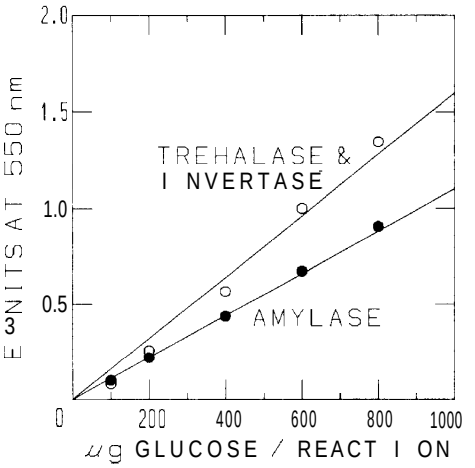

Fig. 5. Standard curve for glucose. Amylase activity was determined after dilution with water (1 : 1 volume) at the end of the reaction with DNSA reagent.

Table 1. Effect of dietary iBMOS and 5-PMOS on the larval weight gain, the gut trehalase, and on the digestive amylase and invertase activity of *M. domestica* 2 days after treatment.

Compound and dietary conc., ppm	Relative to control, % ± SE[a]			
	Δ Weight	Trehalase	Amylase	Invertase
iBMOS				
50	75±14	69±4[b]	101±1	84±1[b]
60	40±5[b]	60±1[b]	81±1[b]	82±0[b]
5-PMOS				
5	61±8[b]	59±0[b]	121±2	105±3
10	30±5[b]	56±1[b]	119±1	107±0

[a] Larval weight gains are averages with their SE values of 5 replicates of 6 test tubes (13 x 150 mm) including 2 larvae each. Larvae l-2 days old (12±1 mg) were introduced into the medium and maintained for 2 days. The enzyme assay data are average of 3 replicates. In the control, larval weight gain was 8.7±0.3 mg, and the trehalase, amylase and invertase activities were 0.73 ± 0.01, 1.50 ± 0.02 and 0.88 ± 0 mg glucose/reaction, respectively.
[b] Differ significantly from the untreated control at $P = 0.05$.

showed no inhibitory effect on the digestive amylase and invertase. Similar phenomena were observed when 50 and 60 ppm iBMOS used. These results indicate that iBMOS and 5-PMOS have at least two actions, the first of which affects AChE activity (Hirashima et *al.,* 1989b), similar to other conventional acyclic organophosphorus compounds at lethal concentrations, and the second of which affects biochemical processes leading to a reduced activity of trehalase at sublethal concentrations. Hence, the trehalase is an important system to assess retardation of growth caused by iBMOS, 5-PMOS and other related compounds.

ACKNOWLEDGEMENT

This work was supported in part by a Grant-In-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and the JSPS (Japan Society for the Promotion of Science) Fellowship to I. Ishaaya for research in Japan.

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