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EFFECT OF MICROSPORIDA INFECTION ON THE ESTERASES ACTIVITIES IN *AGROTIS SEGETUM* CATERPILLARS

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Effect of Microsporida Infection on the Esterases Activities in *Agrotis segetum* Caterpillars. Yefimenko T. M., Sundukov O. V., Issi I. V. — Microsporida infection of *Agrotis segetum* Schiffermüller larvae causes numerous changes of separate fractions activities belonging to carboxylesterase isoenzyme complex. Such shifts are found to correlate with parasite development stages, activities of several fractions being increased early after *Vairimorpha antheraeae* incorporation up to the beginning of sporogony. During intensive sporogony and spore forming the enzyme activities are proved to decrease. Activities of multiple esterase molecular forms at different microsporidiosis stages may be correlated with cell pathology patterns and cell compartments damages taking part in the enzymes synthesis and transportation. These described changes may be also due to such Microsporida-induced pathological features as lipid metabolism and hormone balance impair followed by decreased reproductive function.

Key words: microsporida infection, *Vairimorpha antheraeae,* Noctuidae, *Agrotis segetum,* carboxylesterase isoenzyme complex, lipid metabolism, hormone balance.

Влияние микроспоридиоза на активность эстераз у гусениц озимой совки *Agrotis segetum*. Ефименко Т. М., Сундуков О. В., Исси И. В. — Сопоставлялись уровни суммарной эстеразной активности и активность отдельных эстеразных фракций в брюшных нервных цепочках здоровых и зараженных микроспоридией *Vairimorpha antheraeae* гусениц озимой совки *Agrotis segetum* Schiffermüller. Выявлено, что эстеразная активность увеличивается от момента внедрения паразита в ткани хозяина до начала спорогонии. Период наибольшей активности эстераз соответствует вегетативному размножению микроспоридий, а наименьшей — их массовому спорообразованию. Предполагается возможная взаимосвязь между изменением активности эстераз и такими проявлениями патогенеза микроспоридиоза насекомых, как нарушение липидного обмена, гормонального баланса и последующего снижения репродуктивных функций. Обсуждается сопряженность активности эстераз с особенностями патологии клетки и ее компартментов, учавствующих в синтезе и транспорте этих ферментов на разных этапах микроспоридиоза.

Ключевые слова: патогенез микроспоридиоза, *Vairimorpha antheraeae,* Noctuidae, *Agrotis segetum,* активность эстераз, липидный обмен, гормональный баланс.

Introduction

Pathological process during microsporidiosis infection (MI) in susceptible insects is thought to be due to several factors including exhausting of bioenergetical systems of the host organism; damage of parasites-invaded cells, tissues, and organs; hormonal balance impair; intoxication of the host organism by parasite's metabolic products (Weiser, 1951; Issi, 1986). Some biochemical aspects of host-parasite interactions remain, however, unclear.

Being sure that the changes in enzyme activities which are the main regulative factors of the cell metabolism reflect the depth of the pathological process we have determined esterases activities in the nerve chain of *Agrotis segetum* Schiffermüller (Noctuidae) abdomen on the different stages of MI. The changed esterases activities level might have become a marker of MI caused physiological disturbances specific for insect neural system.

Material and methods

In all the experiments we used *A. segetum* larvae originated from eggs of the same female, ranged and selected according to their weight and age, the ecdysis for the next age having taken place at the same day. They were incubated in entomological tubes and perorally infected by the same quantities of medium containing a known dose of parasite spores. The insects which had not eaten all the food during 12 hs were se-

lected out. The larvae were fed on the medium proposed by Lopatkin et al. (1986) at +24°C, the light day lasted during 18 hs.

We worked with a Microsporida *Vairimorpha antheraeae* (Simtchuk, Lysenko, Tchetkartova, 1979) (Burenellidae) passed a lot of times through *Mamestra brassicae* (Noctuidae) and *A. segetum* larvae. *Antheraea pernyi* Guerin-Meneville is the original host of this parasite (Simtchuk et al., 1979) which is also highly pathogenic for a lot of other Lepidoptera insects.

The time of insect infection was always chosen taking into consideration the necessity to have microsporides of the different developmental stages at the day of analysis. To reach such a situation, 20 larvae in each experiment were infected belonging to the 2nd age and analysed at the 10–15th days post infection (p. i.), next 20 larvae of the 3rd age were infected and analysed in 3–7 days p. i. etc. The infecting doses were 10 times higher for the larvae of the next age comparing to those ones of the former one (100 spores/larva for 2nd-aged larvae, 1000 spores for 3rd-aged ones etc.).

So we had three variants in our experiment: control insects (healthy larvae), those ones with initial stages of the parasite development (in 3–7 days p. i.), and the larvae with progressed disease (in 10–15 days p. i.).

To obtain electrophoretical esterases separation, we used a gel system with tris-veronal electrod buffer (pH 7.0), acrylamide concentration in the separating gel being 7.5% (pH 7.5); this gel contained also Triton X-100 (0.2%).

Each nervous chain was homogenised using 100 μl of 40% sucrose containing 1% Triton X-100. For esterases composition assays, each tube was loaded by 50 μl of the sample; to estimate only choline esterase reaction, we put into each tube 100 μl of the sample. Each experiment was carried out with four parallel samples.

After electrophoresis the gels were incubated in the medium containing 0.56% I-naphtylacetate in 0.2 M phosphate buffer (pH 7.0), 2% acetone, and 0.2% fast blue PP (Šula, Weyda, 1983). Cholinesterase activity was determined with acetylthiocholine iodide as a hydrolized substrate (Karnovsky, Roots, 1964) without any preincubational gel fixation in formol. If the test-toxicant had been used the gels were preincubated in the toxicant-containing medium during 5 min. The samples were then run at the apparatus PEFA-1 using 2 mA current during first 30 min and later 4 mA current.

Kinetic properties of esterases activities were obtained using p-nitropenylacetate (p-NPA) as a substrate (Brick et al., 1977). For each sample, one nervous chain was homogenized in 1 ml of 0.2 M phosphate buffer solution, pH 7.6. The p-NPA-containing incubation medium was prepared immediately before experimant and included homogenate (0.1 ml), 0.2 M phosphate buffer pH 7.6 (0.3 ml), water (0.2 ml), and p-NPA (0.4 ml of the stock 1.5×10^{-3} M solution). The samples were incubated 2–4 min at +20–24°C, their optical densities were measured at 390 nm. Each experimental variant had been tested in three tubes or more.

To determine the stage of Microsporida development and the density of parasite populations in the larvae infected, we counted the average number of parasites under light microscope in stained smears, five fields having been always used for count.

Results

Esterases electrophoresis using acetylthiocholine iodid permits to detect the localization of E_7 and E_8 cholinesterase fractions (fig. 1, *f*). Using I-naphtylacetate we detect according to their localization both non-specific esterases and also multiple molecular esterase forms hydrolyzing acetylthyocholine iodid (fig. 1, *a–e*). The activity of all the non-specific esterase fractions E_1–E_6 being inhibited by diazoxone (10^{-7}–10^{-5} M), we thought these fractions to be carboxylase ones (EC 3.1.1.1.).

The larvae carrying parasites of different developmental stages (parasites proliferation was found at the 3–7th days p. i. and their sporogony and sporogenesis — at the 10–15th days p. i.) possessed different activities of separate carboxylesterase molecular forms. At the 3rd day p. i. (fig. 1, *b*) we found the increase of E_4-fraction activity, at the 7th day (fig. 1, *c*) it was also accompanied by higher activities of other molecular forms of carboxylesterase — E_1–E_3, E_5, E_6.

The microscopic study of infected larvae fat body showed the 1–8-nuclei-containing meronts of the first merogony to be the prevalent form at the 3rd day p. i. At the 5–7th days p. i. we found mostly 1–8-nuclei-containing meronts of the second merogony, sporonts being rather rare (tabl. 1). Fat bodies of such larvae rested white and demi-transparent like these in healthy ones.

At the 13th day p. i. we detected step-wise drop of activities for all the multiple molecular carboxylesterase forms (fig. 1, *d*). In the organisms of larvae survived up to the 15th day p. i. only four molecular forms (E_3–E_6) were detected to express instead of six ones (fig. 1, *e*).

This period is marked with fat body exhausting and cells separation. All the space in the smears is filled with parasite spores.

It should be noted esterases spectrum and esterases activities of control healthy larvae remained without changes during the period studied.

The study of kinetic parameters of abdomen nerve chain esterases in *A. segetum* larvae proved the highest hydrolyzing activity was found in the p-NPA concentration interval 0.2–0.3 mM/l. So the levels of esterases activities for healthy and infected insects were compared in the limits of this interval. Having added eserine to the incubation media (10^{-5}M) we noted no drop of esterase activity; so we suggest that in experiments with enzymatic hydrolysis of p-NPA we detected the activity of izoenzyme carboxylesterase complex. The comparing of hydrolysis curves (fig. 2) showed the activity level of carboxylesterase to be significantly decreased at the 10–13th days p. i.

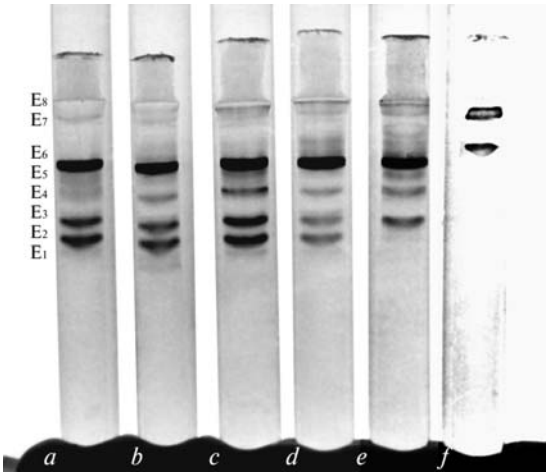

Fig. 1. Composition of esterase fractions in abdominal nerve chain of *A. segetum* caterpillars, healthy and microsporidiosis-diseased ones: *a–e* — the hydrolyzed substrate is I-naphtylacetate; *f* — the hydrolyzed substrate is acetylcholine iodide; *a* — control healthy caterpillars (without infection); *b* — caterpillarsat the 3rd day p. i.; *c* — caterpillars at the 7rd day p. i.; *d* — caterpillars at the 12th day p. i.; *e* — caterpillars at the 15th day p. i.; E_1–E_8 — multiple molecular esterase forms.

Рис. 1. Фракционный состав эстераз в брюшной нервной цепочке здоровых и больных микроспоридиозом гусениц озимой совки: *a–e* — гидролизуемый субстрат I-нафгилацетат; *f* — гидролизуемый субстрат ацетилтиохолиниодид; *a* — контроль (гусеницы без заражения); *b* — 3-й день заражения; *c* — 7-й день заражения; *d* — 12-й день заражения; *e* — 15-й день заражения; E_1–E_8 — множественные молекулярные формы эстераз.

Discussion

Esterases form a polyenzyme complex hydrolyzing ester link of carbonic acids. Nowadays, the classification of non-specific esterases is provisory because each of isolated esterase types possesses identical subunits composition; these subunits form a polymer molecule structure with overlapping substrate and inhibitory specificity (Choudhoury, 1972). Such properties predetermine all the plurality of esterases functions in the animal organisms. In insects these enzymes take part in the mobilization and energetic catabolism of fat substances (Lands, 1965; Ahmad, 1976); they control also juvenile hormone titers (Hammock, 1985) and are important for reproduction and vitellogenesis processes (Hooper, Wan, 1969; Kai, Hasegawa, 1973).

Table 1. Predominant stages of microsporides development on the smears taken from *A. segetum* larvae tissues at the time of biochemical analysis

Таблица 1. Преобладающие фазы развития микроспоридий на мазках из тканей насекомых в сроки проведения биохимических анализов

Days post infection	Stages of parasites development				
	meronts		sporonts	sporoblasts	spores
	I	II			
Control	0	0	0	0	0
3	3,4±0,68	0	0	0	0
5–7	0,8±0,37	5,4±0,89	0,4±0,22	0	0
10–13	0	0	4,1±1,07	0,4±0,24	62,0±10,7
15	0	0	0	0	90,0±7,07

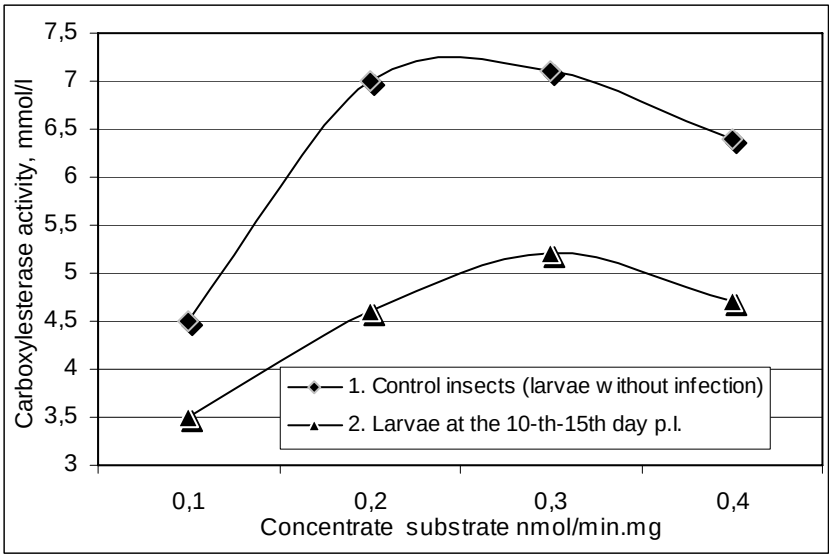

Fig. 2. Carboxylesterase activity in the abdominal nerve chain of Agrotis segetum larvae, healthy and microsporidiosis-diseased ones.

Рис. 2. Активность карбоксилэстеразы брюшной нервной цепочки у здоровых и больных микроспоридиозом гусениц озимой совки.

MI in insects causes the impair of some esterases-controlled physiological functions. It is accompanied by such pathologies as ecdysis and metamorphosis disturbance, decrease of fecondity; the complete castration and decreased resistance against unfavourable environmental factors are more rare events (Issi, 1986).

We have found all the pathological patterns mentioned above in *A. segetum* and *M. brassicae* larvae infected by *V. antheraeae*. If diseased larvae rested alive after metamorphosis, the butterflies from such larvae possessed a low reproductive possibilities (Yefimenko et al., 1990; Yefimenko, 1992). The disturbances of ecdysis, metamorphosis, and diapause are usually due to juvenile hormone titers changed during MI-infection (Issi, 1968; Metspalu, 1975, 1980; Metspalu, Hiyesaar, 1980) because of parasite being able to synthesize this hormone (Fischer, Sanborn, 1962, 1964; Brand, 1972; Street, Brandfield, 1978). The esterases are known to take part in juvenile hormone regulation (Hammock, 1985), so the interaction between hormonal disbalance and esterases activity in insects after MI can be supposed.

It should be noted that host-parasite interactions during infection changed from latent to clear antagonistic ones, these processes being detected both at cellular and whole organism levels.

Non-specific esterases synthesized mostly in granular endoplasmatic reticulum are then processed and go through Golgi apparatus; later they are transported from there to lysosomes (Alberts et al., 1986). The inhibition of host lysosome system was described as a pathological event especially specific for MI (Wiedner, Sibley, 1985; Sokolova, 1989). After Microsporida invasion into the host cell the endoplastic reticulum becomes shifted to the focus of pathogen development, the synthetic reactions being increased (Vavra, 1965; Sprague, Vernik, 1969; Issi, 1983; Sokolova, 1989). In such a way they assure the development of the parasite having no proper mitochondria. The increase of carboxylesterase activity in our experiments may be due to the higher level of synthesis processes during early MI period. At this stage there is also high oxygen consumption by host's mitochondria because of parasite's increased ATP need (Sokolova, 1989). During vegetative development of *V. antheraeae* the body masses and the

intensivity of respiration of Noctuidae larvae increased accompanied by higher resistance to bacterial preparations (Yefimenko, 1989).

The *V. antheraeae* sporulation causes later the host cells damage and disintegration, the cell nuclei and mitochondria remain alive after destruction of other cell organellae (Issi, 1983, Sokolova, 1989); the dropped esterase activity in *A. segetum* larvae at the $10-15^{th}$ days p. i. may be due to such drastic intracellular changes. This stage of host-parasite interactions is also marked with significant loss of larvae body mass, decreased respiration level of insects and of their isolated mitochondria, increased susceptibility to bacterial infections (Yefimenko, 1989; Sokolova, 1989).

So, the increased esterase activities detected in these experiments in *A. segetum* larvae during several early MI stages and the marked fall of such activities at the final stage of the parasite development are patterns characteristic for MI pathogenesis; the "increase of metabolism processes" necessary for complete developmental cycle of a parasite generation is changed by the fall of all the host's physiological functions.

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