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CONTENT OF SACCHARIDES AND ACTIVITY OF α-GLYCOSIDASES IN *GALLERIA MELLONELLA* LARVAE INFECTED WITH ENTOMOPATHOGENIC NEMATODES *HETERORHABDITIS ZEALANDICA*

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ABSTRACT. Concentration of glycogen, maltose, and trehalose as well as the activity of hydrolases catabolising them: α-amylases, glucoamylase, maltase, and trehalase were studied during a 48 h infection of *Galleria mellonella* (Lepidoptera) instar larvae infected with infective *Heterorhabditis zealandica* (20 nematodes/insect). The content of trehalose in the infected insects during the first day of infection was higher ($p < 0.05$) than in the controls. The concentration of maltose and glycogen were similar in both groups despite the fact that the activity of all examined α-glycosidases was generally much higher in the infected insects than in the controls.

Key words: amylase, entomopathogenic nematodes, glycogen, *Heterorhabditis zealandica*, trehalose.

INTRODUCTION

Chemical insecticides are increasingly frequently replaced by biological preparations. In that group gradually increases the share of entomopathogenic nematodes, which are safe for the environment, human health and livestock (Smart 1995).

In the literature, besides papers on immunology, there is little information on the influence of infection with those nematodes on the physiology and biochemistry of the infected insects, including metabolism of sugars (Peterson 1986, Senthamizhselvan and Blackshaw 1990, Jarosz 1998, Żółtowska et al. 2003). In the earlier studies, we have investigated the metabolism of trehalose and glycogen in larvae of *Galleria mellonella* infected with *Steinernema affinis* and *S. feltiae* (Dmitryjuk et al. 2001, Żółtowska et al. 2001). This study aimed at identifying, in the same model organism, the changes in content of three basic sugars: glycogen, trehalose, and maltose, and the activity of hydrolases degrading them during the invasion of an entomopathogenic nematode *Heterorhabditis zealandica* that belongs to Heterorhabditidae. Acquiring knowledge on processes related to the use of saccharides by the infected insects will allow a better understanding of the pathogenesis and explain the consequences of the infection.

MATERIAL AND METHODS

The material for the study consisted of the last larval stage before pupation (instar) of *G. mellonella*. The insects were bread in bee wax. The larvae of the insect were used for both reproduction of the nematode – *H. zealandica* and for the experiment itself. Nematodes were propagated through wax moth larvae (*G. mellonella*). IJs were harvested 14 days post infection and stored in aerated 0.01% formalin at 4°C (Dutky et al. 1964). Instar larvae of *G. mellonella* were divided into two groups: controls (n = 50) and infected (n = 60). Both groups were placed on filtration paper in tightly closed Petri dishes. The experimental group was infected with 5 ml of suspension containing the invasive nematodes. There were 20 *H. zealandica* larvae per one instar. The control larvae were sprinkled with an appropriate quantity of distilled water. During the experiment the insects were not fed. After 0 h, 12 h, 24 h, 36 h, and 48 h from infection, 10 caterpillars from each group were picked at random. They formed the material for further examination. After 48 h since infection, 10 larvae from the experimental group were examined to confirm effectiveness of the infection. The experiment was carried out in triplicate.

Obtaining the extract from larvae of *G. mellonella*. The caterpillars were homogenized in glass Potter homogeniser with 0.9% NaCl at 1: 6 w/v. The homogenate was centrifuged for 15 minutes at 2000 x g. A half of the supernatant obtained was used for determinations of protein and enzymatic activity. The other half was used for marking the saccharides.

Enzymatic identification. Activity of α-amylase was identified by the Caraway's method (1959), that of glucoamylase, trehalase and maltase by Dahlqvist's method (1968) using as substrates the 1% solution of glycogen, 50 mM trehalose and 50 mM maltose respectively. The incubation was carried for 1 h at 37°C, using 0.07 M veronal-acetate buffer with pH 7.5; 5.0; 4.6, or 6.5, respectively for α-amylase, glucoamylase, trehalase, and maltase.

Measurement of carbohydrates contents. In the extract, the enzymatic proteins were precipitated by five-minute denaturation at 100°C and centrifuging for 10 min. at 2000 x g. In the supernatant obtained the content of glycogen was identified using Solling and Esmann method (1975), trehalose using the enzymatic method according to Kienle et al. (1993), maltose, after chromatographic separation in the system of n-butanol/acetic acid/water at 12/3/5, using the method by Park-Johnson (after Lisowska 1970).

The content of protein was measured according to Bradford (1976). The results were processed by Students t-test.

RESULTS

The infection was effective. The average extensity of the infection was 84.66% (range 74-100%), and intensity 104.3 (range 84-125 nematode/larva).

The results presenting the concentration of carbohydrates are provided in Table 1. Similar trends in glycogen level changes were observed in both the controls and the infected groups. At the 12 h of the test the saccharide content in both groups was higher than at the beginning of the experiment. During the following day its level decreased to increase again after 48 h. The content of glycogen in all cases was higher in the controls than in the infected group but the differences between them were insignificant. Also no significant differences in concentration of maltose between the controls and the infected group were observed (Table 1). The lowest level of that disaccharide was found in both groups of larvae at the 36 h of the experiment. The largest differences were observed in case of trehalose (Table 1). In the controls, during the initial 12 hours of the experiment, the concentration of trehalose decreased rapidly and then an increase of sugar was observed. Also in the infected insects a decrease in content of trehalose was observed after 12 h post infection but it was less significant than in case of the controls. During the following twelve-hour periods a continuous decrease in the level of trehalose by ca. 30-40% was observed in the infected larvae. It should be noted, however, that the level of trehalose in infected insects during the first day after infection was significantly higher than in case of the controls (Table 1).

Table 1. The concentration of saccharides from larvae of *Galleria mellonella* infected with *Heterorhabditis zealandica* (mg/g)

Time (h)	Group					
	Control			Infected		
	Glycogen	Maltose	Trehalose	Glycogen	Maltose	Trehalose
0	5.68 ± 1,95	4.11 ± 0.58	5.84 ± 3.17	–	–	–
12	6.78 ± 1.34	3.73 ± 2.31	0.23 ± 0.19	6.24 ± 1.25	3.77 ± 2.83	1.67 ± 0.56*
24	6.03 ± 0.81	4.29 ± 1.02	0.35 ± 0.20	5.09 ± 0.22	4.09 ± 1.59	0.98 ± 0.28*
36	3.80 ± 0.45	1.83 ± 0.31	0.71 ± 0.68	3.32 ± 0.66	2.12 ± 0.14	0.73 ± 0.70
48	5.26 ± 0.77	2.32 ± 0.39	0.86 ± 0.56	4.50 ± 0.71	2.59 ± 0.90	0.49 ± 0.36

The activity of the major enzyme in metabolism of glycogen – α-amylase was in all cases higher in the infected insects than in the controls (Table 2). The infected *G. mellonella* had the activity of enzyme more than twice higher than in the controls after 24 h infection. The differences between the mean values for those groups were statistically significant. Also, as of 24 h after infection, the activity of glucoamylase was significantly higher ($p < 0.05$) in the infected insects than in the controls.

The activity of maltase in the control group in the 12 h was twice and in the 24 h three times lower than at the beginning of the experiment (Table 2). In the infected group the decrease of that enzyme's activity was minimal. Differences between the mean values for the groups were at that time significant. During the following

Table 2. The activity of enzymes of saccharide metabolism from larvae of Galleria *mellonella* infected by *Heterorhabditis zealandica*

Time (h)	Group: Control				Group: Infected			
	α-amylase (U/mg)	Glucoamylase (μmol/mg)	Maltase (μmol/mg)	Trehalase (μmol/mg)	α-amylase (U/mg)	Glucoamylase (μmol/mg)	Maltase (μmol/mg)	Trehalase (μmol/mg)
0	49.1 ± 14.6	389.5 ± 12.7	225.2 ± 55.9	117.8 ± 50.3	–	–	–	–
12	33.7 ± 7.6	31.3 ± 10.4	108.3 ± 16.3	66.7 ± 20.9	38.3 ± 16.2	33.2 ± 21.9	189.3 ± 30.8*	94.3 ± 29.4
24	20.5 ± 13.7	98.2 ± 31.1	78.1 ± 29.1	86.6 ± 39.9	48.6 ± 17.9	184.3 ± 37.5*	160.4 ± 21.4*	100.3 ± 6.8
36	71.8 ± 38.7	138.6 ± 32.4	157.0 ± 13.9	126.8 ± 60.7	162.3 ± 16.2*	200.2 ± 57.3*	132.7 ± 12.9	100.3 ± 25.3
48	100.5 ± 25.1	139.4 ± 40.2	168.8 ± 15.6	102.4 ± 41.7	209.2 ± 32.1*	191.7 ± 76.5	119.8 ± 21.4	105.3 ± 17.5

hours in the infected group a continuous decrease in activity of maltase was observed while in the controls it was the opposite and its activity increased. Comparing the activity of trehalase in both groups its almost stabile level in the infected group should be noticed while in case of the controls the activity of the enzyme during the first day of the experiment was at a decreased level (Table 2). As a result of trehalase activity in the infected *G. mellonella* was higher at that time than in the controls. During the second day the activity of trehalase in both groups was similar.

DISCUSSION

Analysis of the results obtained for the controls indicates that the starvation of *G. mellonella* leads, as expected, to clear changes, first of all in the contents of such saccharides as glycogen and trehalose. During the initial 12 hours without food not only decrease of trehalose but also, which is interesting, a parallel increase in the concentration of glycogen was observed (Table 1). A similar phenomenon was appeared in starved larvae of parasitic nematode *Anisakis simplex* (cf. Łopieńska 2004). We suspect that trehalose is a sugar metabolised first not only in the nematodes but also by larvae of insects. A part of glucose released from trehalose may serve to resynthesis and maintenance the stocks of glycogen at a relatively stabile level, at least during the initial 24 h of starvation (Table 1).

Comparisons of saccharides concentration in the controls and the infected group leads to very interesting observation. They indicate that infection of *G. mellonella* instar larvae with *H. zealandica* nematodes limits the changes caused by starving the insects. It is particularly well visible in case of trehalose and applies to a lesser degree to contents of glycogen. For example, at the 12 h in the control the concentration of trehalose was 20 times lower than at the beginning of the experiment while in the infected insects only 3 times lower. On the other hand the level of glycogen in the controls increased by 20% while in the infected insects by around 10% only (Table 1).

It is difficult to present a clear interpretation of those results as in the infected *G. mellonella* the activity of enzymes decomposing the studied saccharides was generally higher than in the controls (Table 2). Both those facts, the lower reduction in the levels of sugars and a clearly higher activity of the enzymes degrading them, indicate indirectly a more intensive development of the process of synthesis of the studied saccharides in the *G. mellonella* larvae infected with *H. zealandica*. That suggestion stands in opposition to the findings of Senthamizhselvan and Blackshaw (l990). Those authors observed a significant decrease in the use of oxygen indicating a slow down in general metabolism after infecting the same host with *H. heliothidis*. On the other hand, Peterson (1986) described the reduction in glycogen and trehalose as well as protein contents in mermithid-parasitised simuliids. We

obtained results similar to those by Peterson (1986) in the earlier study, where the larvae of *G. mellonella* were infected with *Steinernema affinis* nematode (Dmitryjuk et al. 2001). The level of trehalose and the activity of trehalase were slightly lower in the infected larvae than in those not infected. The results obtained in the current experiment were different. The content of trehalose in the infected caterpillars during the first day of the infection was significantly higher than in the controls (Table 1). As mentioned earlier, that could suggest intensification of trehalose synthesis, known to be an anti-stress factor in invertebrates (Behm 1997).

The differences between the results of our two studies may be explained in two ways. First, by using the host in a different development stage. In the experiment by Dmitryjuk et al. (2001) the younger 3-th larval stage of *G. mellonella* was infected than in the current study. Second, the caterpillars were infected with species belonging to different families. That second difference should be considered more significant. We believe that because in our other study we observed a decrease in the level of glycogen and a decrease in amylase activity in the instar larvae of *G. mellonella* infected with *S. feltiae* (cf. Żółtowska et al. 2001). Those differences cannot be explained by the levels of enzymatic activity of parasitoids used for the infection because, as established, the activity of α-glucosidases from *H. zealandica*, *S. feltiae*, and *S. affinis* was similar (Żółtowska and Łopieńska 2003). As a consequence, it can be assumed that the mechanisms regulating the metabolism of saccharides in *G. mellonella* probably were modified in different ways after infection with entomopathogenic nematodes belonging to Heterorhabditidae family and in a different way in case of infection with Steinernematidae.

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