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Content of glycogen and trehalose and activity of α-amylase and trehalase in *Galleria mellonella* larvae infected with entomopathogenic nematodes *Steinernema affinis* and *S. feltiae*

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ABSTRACT. **Introduction**. The influence of infection with two species of entomopathogenic nematodes of Steinernematidae family on metabolism of glycogen and trehalose of the host was studied. **Material and methods**. Last instar larvae (L_7) of *Galleria mellonella* were experimentally infected with *Steinernema affinis* and *S. feltiae*. At 6, 12, 18 and 24 h after infection concentrations of trehalose and glycogen as well as activity of trehalase and α-amylase were determined. **Results**. The content of glycogen was lower in insects infected with *S. feltiae* than in the controls and animals infected with *S. affinis*. The content of trehalose was higher in insects from both infected groups than in the controls. Its concentration was slightly higher in larvae infected with *S. affinis* than in those infected with *S. feltiae*. The activity of α-amylase after infection with *S. affinis* was low. It was significantly higher in insects infected with *S. feltiae*. In animals of both infected groups, following a significant reduction at 6 h, the activity of trehalase remained at a similar level, higher than in the controls. In the paper the effects of infection with (i) different species of entomopathogenic nematodes and (ii) the importance of the developmental stage of the insect-host for changes in its metabolism of glycogen and trehalose were discussed.

Key words: amylase, entomopathogenic nematode, glycogen, trehalase, trehalose.

Introduction

Entomopathogenic nematodes are used widely for biological control of harmful insects [1]. The literature still offers little information on their influence on metabolism of the infected insect [2–7]. Peterson [7] described the reduction in protein as well as glycogen and trehalose contents in mermithid-parasitised simuliids. In our earlier studies we noticed disturbances in metabolism of carbohydrates in the host organism appearing as a consequence of the infection with *Steinernema* [4–6]. The most intensive changes were observed during the first 24 h after infection, therefore in this study the level of glycogen and trehalose, the two most important sugars for the insects [8], and major hydrolases metabolizing them — trehalose and α-amylase were monitored at 6 h intervals. The model organism — *Galleria mellonella*, used not only for determination of the degree of infectivity of individual species of entomopathogenic nematodes but also for breeding the nematodes for both research and commercial purposes was used as the host [1, 9].

Materials and methods

The material for the study consisted of the last larval stage (L_7) of *G. mellonella*. The insects were reared on bee wax in the dark at 22°C. Caterpillars of *G. mellonella* were used for both reproduction of the nematodes — *S. affinis* and *S. feltiae* and for the experiment itself. Nematodes were propagated through wax moth larvae. Invasive juveniles were harvested 14 days post infection and stored in aerated 0.01% formalin at 4°C [9].

The larvae of *G. mellonella* were divided into

three groups: controls (n = 50), infected with *S. affinis* (n = 60) and infected with *S. feltiae* (n = 60). Each group was placed on wet filtration paper in tightly closed Petri dishes. The insects were infected by spraying with 5 ml of solution containing invasive larvae of entomopathogenic nematodes prepared in a way giving *ca* 20 nematodes per larva. The control larvae were sprinkled with an appropriate quantity of distilled water. During the experiment the insects were not fed. After 0, 6, 12, 18 and 24 h from infection 10 caterpillars from each group were picked at random. They formed the material for further examination. After 24 h and the 5-th day since infection 10 larvae from the both infected groups were examined to confirm effectiveness of the infection. The experiment was carried out in triplicate.

Obtaining the extract from larvae of *G. mellonella*: Caterpillars were homogenized in glass Potter homogenizer (1:6 w/v) with 0.9% NaCl. The homogenate was centrifuged for 15 minutes at 2,000 *x g*. A half of the supernatant obtained was used for determinations of protein and enzymatic activity. The other half was used for analyse of saccharides.

Enzymatic analysis: Activity of α-amylase was identified by the Caraway's method [10], trehalase by Dahlqvist's method [11]. The incubation was carried for 1 h at 37°C, using 0.07 M buffer of veronal — acetate with pH 5.6 and 4.6 respectively for α-amylase and trehalase.

Measurement of carbohydrates contents: In the extract of larvae proteins were precipitated by five-minute denaturation at 100°C and centrifuging for 10 min. at 2,000 *x* g. In the supernatant obtained the content of glycogen was identified using Sølling and Esmann method [12]. HPLC was used for determination of trehalose content. The separation was carried out on Rezex RMN Carbohydrate Na^+ column (30 x 0.78 cm) at the flow rate of deionized water of 0.4 ml per minute, in a Shimatzu chromatograph with a refractometric detector.

The content of protein was measured according to Bradford [13]. The results were processed by Students t-test.

Results

After 24 h lasting infection *S. affinis* was found at the average number of 6.4 nematodes per larva in 46% of the hosts, and *S. feltiae* at the average number of 9.2 nematodes per larva in 53.3% of the hosts. The examinations repeated after 5 days of invasion gave much higher indexes of the infection and confirmed that it was effective. The average prevalence were 48.33% (range 40–60 %) and 57.08 % (range 52–72%), and the intensity were 88.6 nematode/larvae (range 66–110) and 104.2 nematode/larvae (range 80–150) for the infection with *S. affinis* and with *S. feltiae* respectively.

The data concerning glycogen and trehalose content are presented in Table 1. The content of glycogen in larvae of the control group and those infected with *S. affinis* was at similar levels during the course of the experiment. Only at 18 h after infection a significantly higher level of glycogen in the infected insects ($p < 0.05$) was observed. Differences were also found in glycogen concentration between groups of the hosts infected with *S. affinis* and *S. feltiae*. The content of glycogen in insects infected with *S. feltiae* was always lower than in those infected with *S. affinis*, and also lower than in the controls (Table 1). A higher than initial level of glycogen in all groups at 6 h of the experiment should be noticed (Table 1). On the other hand, the content of trehalose in the insect at that time was significantly lower than that at the start of the experiment. That decrease was much more pronounced in the controls than in larvae infected with entomopathogenic nematodes (Table 1). The lowest level of trehalose was observed in control *G. mellonella* at 12 h, while in the infected larvae the concentration of trehalose decreased gradually during the course of the experiment. In all cases, the content of trehalose in extracts from insects infected was higher than in those of controls. What is more, the concentration of that disaccharide was always slightly higher in larvae infected with *S. affinis* than in those infected with *S. feltiae* (Table 1).

The activity of α-amylase, the enzyme hydrolyzing glycogen, in nematodes was lower in *G. mellonella* infected with *S. affinis* than in the controls, however a significant decrease of that enzyme activity ($p < 0.05$) was recorded only at 18 and 24 h (Table 1). In insects infected with *S. feltiae* the activity of α-amylase was significantly higher than in infected with *S. affinis* and the controls (Table 1).

The activity of trehalase decreased gradually in the controls during the course of the experiment. On the other hand in insects of both infected groups, following a significant decrease at 6 h after infection, activity of the enzyme remained at a similar level. At 6 and 12 h activity of trehalase was lower, while at 18 and 24 h it was higher than in the controls.

Table 1. The concentration of glycogen and trehalose and activity of a-amylase and trehalase in the extracts from *Galleria mellonella* infected with *Steinernema affinis* and *S. feltiae*

Time after infection (h)	Control group (a)	Group infected with *S. affinis* (b)	Group infected with *S. feltiae* (c)
	Glycogen (mg/g fresh tissue)		
0	5.52 ± 0.20		
6	6.30 ± 1.06*	6.73 ± 0.31	5.84 ± 0.30
12	4.99 ± 0.97	5.05 ± 0.93	3.41 ± 0.66
18	5.07 ± 0.15	7.12 ± 0.11^{ac}	3.99 ± 0.06
24	5.27 ± 0.78	5.39 ± 0.21	3.28 ± 0.66^{ab}
	Trehalose (mg/g tissue)		
0	4.60 ± 0.61		
6	1.50 ± 0.42	2.29 ± 1.06^{a}	2.08 ± 0.92^{a}
12	1.35 ± 0.52	2.29 ± 0.63^{a}	1.87 ± 1.02
18	1.56 ± 1.07	2.19 ± 1.26	1.74 ± 0.12
24	1.54 ± 0.04	1.86 ± 0.53	1.45 ±0.39
	Activity of α-amylase (U/mg protein)		
0	35.6 ± 1.20		
6	49.5 ± 15.6	40.4 ± 11.4	23.0 ± 3.7^{ab}
12	50.7 ± 2.6	40.7 ± 6.8	79.0 ± 2.3^{ab}
18	50.8 ± 8.0	23.7 ± 12.7^{a}	72.7 ± 1.3^{ab}
24	42.0 ± 2.0	20.8 ± 1.6^{a}	61.4 ± 2.3^{ab}
	Activity of trehalase (U/mg protein)		
0	3.58 ± 0.72		
6	3.41 ± 0.41	1.61 ± 0.50^{a}	0.83 ± 0.19^{ab}
12	2.59 ± 0.66	2.28 ± 0.35	1.64 ± 0.69
18	1.83 ± 0.37	2.39 ± 0.20	2.79 ± 0.42
24	1.63 ± 0.32	2.56 ± 0.19^{a}	2.48 ± 0.98

$^{a\,b\,c}$ — significant difference between means of group ($p < 0.05$), *mean ± SD

Discussion

On the basis of low invasion ratios at 24 h we assume that neither the content of sugars in parasites' tissues nor the activity of their hydrolytic enzymes present in homogenates used for the analysis had any major influence on the results obtained and they may be omitted [see 14 and 15].

In our earlier paper [6] concerning the influence of *Heterorhabditis zealandica* on metabolism of sugars of *G. mellonella* it was suggested that the effect of infection might depend on the development stage of the host and the species of enthomopathogenic nematodes used for infection. That hypothesis was a consequence of comparison between the results of that study and the results of the experiments preceding it [4, 5], where the experimental material consisted of younger stage (L3) of *G. mellonella* infected with *S. affinis* [4] or L_7 larvae infected with *S. feltiae* [5]. In the case of this study it was decided to repeat the experiments using the two earlier used species of entomopathogenic nematodes, *S. affinis* and *S. feltiae*, belonging to the same Steinernematidae family, on L_7 larvae of *G. mellonella*. In this way the results could be used for analysis leading to the conclusions confirming or negating the above suggestion.

Studies of relations between the developmental stage of host and the effects of infection of entomopathogenic nematodes showed a different response of trehalose metabolism of L_3 and L_7 larvae of *G. mellonella*. The infection with *S. affinis* did not change the concentration of that sugar in younger larvae [4], while in the older ones, as shown in this paper, it lead to maintaining trehalose at a higher level than in the uninfected animals. Also the activity of trehalase, with the exception of hours 6 and 12, was clearly higher in older infected larvae than in the controls (Table 1). A different result was obtained for the younger stage (L_3) of *G. mellonella* where the activity of trehalase slightly decreased as a result of infection [4]. As a consequence, it was

concluded that infection with *S. affinis* did not lead to significant changes in the metabolism of trehalose in *G. mellonella* [4]. On the basis of the results of this study we should state that the above conclusion is correct only for the L_3 larvae. For the older larvae stages the influence of infection on the host's trehalose level may be significant. It is particularly well visible in L_7 larvae during the first initial 6 h after infection, and might be related to a significantly decreased activity of trehalase during that time (Table 1).

A relation between the species used for infection and carbohydrates' metabolism of the host can also be noticed, although it is much more difficult to interpret. We expected that the differences in the consequences of infection with nematodes belonging to two different families, Heterorhabditidae and Steinernematidae, would be larger than between effects of the infection with nematodes belonging to the same family. It really was so in the case of trehalose metabolism. The trehalose concentrations obtained during this study for groups infected with *S. affinis* and *S. feltiae* did not differ significantly, although in the first case they were slightly higher (Table1). Results similar in direction of changes were obtained after infecting the insect with *H. zealandica* [6]. It should be noticed, however, that the infection with this nematode belonging to Heterorhabditidae, reduced the consumption of trehalose larger than it was observed in this study during infection with nematodes belonging to Steinernematidae. Different results were obtained in the case of glycogen. The level of glycogen in *G. mellonella* infected with *S. affinis* was much higher than in those infected with *S. feltiae*. What is more, in the case of the later group a similarity to the situation after infection *G. mellonella* with *H. zealandica* was observed [6]. The content of glycogen in insects infected with these two species of nematodes was lower than in the controls. The low level of glycogen in *G. mellonella* infected with *S. feltiae* or *H. zealandica* was correlated with higher activity of host's α–amylase. The activity of that enzyme was *ca* 20-50% higher in larvae infected with *S. feltiae* than in the controls (Table 1), while in infected with *H. zealandica* it was twofold higher [6].

It can be concluded on the basis of results obtained from this and our earlier studies [4–6] that infection of insects with entomopathogenic nematode influences glycogen and trehalose catabolism in the host. The magnitude of changes in metabolism of carbohydrates depends on the development stage of the host and the species of nematodes used for the infection.

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