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Activity of superoxide dismutase in *Galleria mellonella* larvae infected with entomopathogenic nematodes *Steinernema affinis* and *S. feltiae*

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ABSTRACT. **Background**. The influence of infection with two species of entomopathogenic nematodes of Steinernematidae family on the activity of superoxide dismutase (SOD) of the host was studied. **Material and methods**. Last instar larvae of *Galleria mellonella* were experimentally infected with *Steinernema affinis* and *S. feltiae* at 20 invasive juveniles per insect. At 6, 12, 18, 24 and 36 h after infection activity of SOD was determined in extracts from infected and control insects. **Results**. The activity of SOD decreased gradually in the controls during the experiment. The activity of enzyme was 2-4-times higher in insects from both infected groups than in the control. During the first 12 h of infection the activity of SOD in insects infected with *S. feltiae* was higher than in those infected with *S. affinis*, then the activity of enzyme in the insects of both infected groups stayed at a similar level. A significant decrease of SOD activity in infected was recorded in second day of the infection.

Key words: entomopathogenic nematode, *Galleria mellonella*, oxidative stress, superoxide dismutase.

Introduction

Reactive oxygen species (ROS) are generated by all organisms during metabolic processes occurring under aerobic conditions. Their quantity increases as an effect of both abiotic (radiation, climatic factors) and biotic stressors [see 1]. That later group includes pathogenic factors such as viruses, bacteria and parasites.

It has been proven that ROS play a significant role in the defensive reactions of host during invasive diseases. Increased synthesis of superoxide radical anion (O_2^-) and hydrogen peroxide was observed after infecting rat and mice with *Fasciola hepatica* [2], during trichinellosis [3, 4] and malaria [5]. In vertebrates, phagocytes generate O_2^-.as a product of one-electron reduction of molecular oxygen by NADPH oxidase in the process called respiratory burst [1]. As a consequence of the damaging influence of ROS on the most important biopolymers of cells such as DNA, membrane lipids, enzymatic and receptor proteins, the respiratory burst of the host's phagocytic cells must be controlled. ROS generated during it are removed by antioxidant systems. Antioxidant enzymes, including superoxide dismutase (SOD), play the basic role in these reactions [1]. It has been proven that antioxidant enzymes occur not only in vertebrates but also in invertebrates, including insects [6–8].

In the available literature no information was found on oxidative stress during the infection of parasitoids. Entomopathogenic nematodes used for control of insects harmful for crops are parasitoids lethal to the insects [9]. It seemed interesting to investigate whether during infection of insects by entomopathogenic nematodes the activity of their antioxidant enzymes changes and, if yes, what is the direction of such change. Does activity stimulation of the host's antioxidant enzymes occur and how strong that reaction could be? This paper focuses on

SOD, one of the most important antioxidant enzymes. The presented study aimed at acquiring knowledge on its activity changes during infection of two entomopathogenic nematode species *Steinernema affinis* and *S. feltiae*.

Materials and methods

The material for the study consisted of the last larval stage (L_7) of *G. mellonella*. The material handling methods, insects and nematodes breeding conditions were identical as during the earlier research [10].

The larvae were divided into three groups (n = 60); controls, infected with *S. affinis*, and infected with *S. feltiae*. 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, 24 and 36 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 both infected groups were examined to confirm effectiveness of the infection. The experiment was carried out in triplicate.

In order to obtaining the extract from larvae of *G. mellonella* caterpillars were homogenized separately in glass Potter homogenizer with 0.9% NaCl (1:4 w/v). The homogenate was centrifuged for 15 minutes at 1,500 *x g*. The supernatant obtained was used for determinations of protein according to Bradford [11] and SOD activity by Beauchamp and Fridovich [12]. The results were processed by Students t-test.

Results

The infection of *G. mellonella* larvae was effective. The prevalence was 68.3% and 72.1% while intensity 10.3 and 11.0 nematodes per larvae after infection with *S. affinis* and *S. feltiae* respectively.

The activity of superoxide dismutase in the larvae of control insects decreased gradually during the experiment and at 36 h it was 3 times lower than at the start (0 h).

Comparing SOD activity in the infected and control larvae (Table 1), it can be noticed that activity of the enzyme was much lower in the controls than in the infected ones. The differences between the discussed average values for those groups were statistically significant ($p>0.05$). Only at 6 h after infection of *G. mellonella* with *S. affinis* no differences in SOD activity were recorded.

The SOD activity in the insect larvae infected with both entomopathogenic nematode species was the highest at 12 h after infection. During the next 12 h it decreased by *ca* 50%. The largest reduction in SOD activity was observed in them at second day after infection (Table 1). The enzyme was more active in insects infected with *S. feltiae* than in those infected with *S. affinis* until 12 h after infection, especially at 6 h ($p < 0.05$). During the later lasting of the experiment (18, 24 and 36 h) no significant differences in SOD activity between groups infected with *S. affinis* and *S. feltiae* were observed.

Discussion

The participation of O_2^- and other ROS in immunological defense is studied increasingly extensively not only in case of vertebrates but also in insects [13–17]. On the other hand there are some data available on their role in defense of the host insect against parasites [16–18]. In the paper by Whitten et al. [18] an increased level of O_2^- and NO after infecting *Rodnius prolixus* with two strains of *Trypanosoma rangeli* was reported. The authors of that paper drew indirect conclusions concerning increased activity of enzymes responsible for formation of those radicals as a consequence of parasite presence. On the other hand, they did not deal with enzymes transforming free radicals, such as SOD.

The ability to produce free radicals, including O_2^-, by last instar larvae of *G. mellonella* was shown by Slepneva et al. [19]. According to those authors the activity of endogenous SOD in hemolymph of that insect was very high. Our studies confirmed that suggestion. SOD activity in the larvae was high at the beginning of the experiment (3.78 U/mg), but during starving the larvae for an extended time (over 12 h) it decreased 2–3 times (Table 1).

Infection of the larvae with entomopathogenic nematodes was the cause of significantly higher SOD activity as compared to the controls. The rapid increase of the enzyme activity in the case of insects infected with *S. feltiae* was observed 6 h after infection. In the case of *G. mellonella* infected with *S. affinis* that moment was shifted in time until 12 h of

Table 1. The activity of superoxide dismutase SOD (mU/mg protein) in L_7 larvae of *Galleria mellonella* infected with *Steinernema affinis* and *S. feltiae*

Time after infection	Control group	Group infected with	
		S. affinis	*S. feltiae*
(h)	a	b	c
0	3.78 ± 0.47*		
6	3.76 ± 0.80[c]	4.18 ± 0.59[c]	9.50 ± 0.87
12	2.97 ± 0.56[b,c]	11.23 ± 1.22	12.95 ± 2.73
18	1.48 ± 0.11[b,c]	9.08 ± 0.65	8.71 ± 0.55
24	1.35 ± 0.33[b,c]	6.94 ± 1.23	6.22 ± 1.33
36	1.15 ± 0.47[b,c]	3.36 ± 0.61	2.87 ± 0.56

[a,b,c] — $p<0.05$ between groups, * — mean ± SD

infection (Table 1). During the later hours of the experiment no significant differences in SOD activity between insects infected with *S. affinis* and *S. feltiae* were observed. Relatively small differences in the enzyme activity between the two infected groups could result from the facts that the infection indicators were similar in both groups and/or that the species used for the infection belonging to the same Steinernematidae family might have caused similar host's defense reactions. It can be inferred that the quantity of O_2^-.formed as a result of infection was similar, but probably not identical, because SOD activity in insects infected with *S. affinis* and *S. feltiae* was slightly different in the course of the infection (Table 1).

This paper shows that infection of *G. mellonella* last instar larvae with entomopathogenic nematodes of Steinernematidae family involves oxidation stress appearing probably as the insect's defensive reaction. At that time the activity of SOD removing superoxide radicals increases evidently. That is particularly well visible during the initial 12 h after infection. We suggest that such a development is a consequence of still good condition of the host at that time [10], which allows it both immunological defense against the parasites and effective protection of own tissues against free radicals formed as a consequence of that infection.

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