

HELMINTHOLOGIA, 54, 1: 3 - 10, 2017

**Adsorption and inactivation of proteolytic enzymes
by *Triaenophorus nodulosus* (Cestoda)**

G.I. IZVEKOVA*, T.V. FROLOVA, E.I. IZVEKOV

I.D. Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Nekouzskii raion,
Yaroslavskaia oblast, 152742, Russia, *E-mail: izvekov@ibiw.yaroslavl.ru**Article info**Received September 20, 2016
Accepted December 15, 2016**Summary**

The proteolytic activity in washings off the *Triaenophorus nodulosus* cestode tegument and the ability of the worms to inactivate proteolytic enzymes were studied. It was found that the major proteolytic activity in the washing samples is represented by the easily desorbed fraction most probably characterizing the activity of the host's enzymes. Serine proteinases are an essential part of these enzymes. It was shown that the worms' incubation medium and their homogenates can inhibit host proteinases and commercial trypsin samples. Suppressive activity of the incubation medium suggests that the inhibitors are rather spontaneously produced by the worms than induced by the presence of proteinases in the surrounding medium. The inhibitor produced by the cestode is hypothesized to be trypsin-specific.

Keywords: fish; Cestodes; *Triaenophorus nodulosus*; digestive enzymes; proteolytic activity; proteinase inhibitors

Introduction

The intestine of vertebrates is an ideal environment for cestodes dwelling there, however, the tapeworms are constantly affected by destructive proteolytic enzymes. Intestinal parasites are less affected by the host's immune systems, but they should resist the action of digestive enzymes. One of the basic mechanisms protecting the tapeworms from the host's proteinases is inhibitor secretion (Soprunov, 1987; Pappas & Uglem, 1990). The concept that parasites can use proteinase inhibitors to survive in the host is over 100 years old. Besides, it is becoming obvious that parasite inhibitors may control the host's immune response (Dzik, 2006; Knox, 2007). Proteinase inhibitors, as well as proteinases themselves, play an important role in the life cycle of parasites, their virulence and pathogenesis (Li *et al.*, 2006; Hwang *et al.*, 2009; Molehin *et al.*, 2012; Rascón & McKerrow, 2013). Inhibitors are produced by parasites to avoid proteolysis by host proteinases and thus survive. It is supposed

that the specificity of parasites to hosts depends on the parasite proteinase inhibitors (Hawley & Peanasky, 1992).

Ample research is devoted to proteinase inhibitors in a wide range of nematodes (for a review, see Knox, 2007). Similar studies focusing on cestodes are scarce; most papers were published long ago and mainly concern *Hymenolepis diminuta* or *H. microstoma* (Pappas & Read, 1972a; Pappas, 1978; Schroeder & Pappas, 1980; Schroeder *et al.*, 1981; Pappas, 1987; Pappas & Uglem, 1990). In some cases the obtained data are contradictory. While inhibitors of all classes of proteinases (aspartyl, serine, cysteine and metalloproteinases) occurring at various stages of parasite life cycle have been discovered in nematodes (Hawley, Peanasky, 1992; Kageyama, 1998; Delaney *et al.*, 2005; Knox, 2007), only inhibitors of serine proteinases (trypsin and chymotrypsin) have been found in cestodes (Matskási, 1984; Pappas, Uglem, 1990; Li *et al.* 2006) so far. It may be due to the differences in the life cycle of these groups of helminths and, consequently, the barriers bro-

* – corresponding author

ken by the parasites to get into the host, as well as to insufficient information on inhibitors in cestodes.

Besides, attempts to detect inhibitor activity in cestodes or extract their proteinase inhibitors are not always successful. Thus, no proteinase inhibitors were found in *Skrjabinia cesticillus* from the chicken intestine and in *Moniezia benedeni* and *M. expansa* from the sheep intestine (Klimenko & K̆eniņa, 1971), and live cestodes *M. expansa* did not inhibit casein hydrolysis (Dubovskaya, 1973). Therefore it was supposed that the proteolytic enzyme inhibitor excreted by the helminths is not active enough to provide survival of worms (Shishova-Kasatochkina & Leutskaya, 1979). The negative results of proteinase inhibitor research in the above-cited papers are probably caused by imperfect methods, which in some cases is admitted by the authors (Klimenko & K̆eniņa, 1971).

There are very few studies devoted to the ability of cestodes inhabiting the fish intestine to suppress host proteinases. It is reported that intact *Proteocephalus longicollis* from salmonids secretes the trypsin inhibitor into the surrounding medium (Reichenbach-Klinke & Reichenbach-Klinke, 1970). It was ascertained that extracts from *Bothriocephalus acheilognathi* larvae and adult worms suppress the trypsin and chymotrypsin activity of the carp intestine *in vitro* (Matskási, 1984).

The present study was undertaken to evaluate the possibility that *Triaenophorus nodulosus*, a cestode common in pike, *Esox lucius*, can inactivate proteolytic enzymes.

Material and Methods

Study objects

The study was performed on cestodes *Triaenophorus nodulosus* (Pallas) from the intestine of pike, *Esox lucius* L. The tapeworms were taken from 17 pikes 310 – 680 mm long caught in the Rybinsk reservoir (Russian Federation, 58°22'30''N 38°28'04''E) during the spring season. In a single pike, the number of helminths was 2 – 4 and their total weight varied from 0.27 to 2.11 g per an individual fish. The fish were caught by gill nets, transported to the laboratory in special containers with water, and immobilized by a blow on the head. The dissection of fish and their intestines, extraction of worms and sample preparation were performed on the ice bath.

Sample preparation

The fish intestines were dissected, the worms were extracted, randomly grouped into samples of similar weight (0.4 – 0.7 g each), and then transferred to 10 ml of Ringer's solution (pH 7.5) for cold-blooded animals. In total, 23 groups of worms were studied ($n = 23$). To remove the host enzymes adsorbed on the cestode surface, the worms were shaken for 30 seconds in Ringer's solution, then transferred to fresh solution twice and shaken for 15 min each time. As a result, three fractions of washings off the tegument were obtained: F_1 , F_2 and F_3 . A total of 7 groups of worms were used when measuring proteolytic activity in different fractions ($n = 7$).

Further, the worms were transferred to 10 ml of fresh Ringer's solution and incubated for 24 hours at a temperature of 10°C. During the entire incubation the worms remained alive. Five groups of worms were used to obtain the incubation medium ($n = 5$). After the incubation, the pH values were measured in the medium.

Next 11 groups ($n = 11$) of worms were homogenized, and the homogenate was diluted with Ringer's solution in the mass-volume ratio of 1:9.

Besides, the homogenate of the pike intestine mucosa was prepared. For this purpose, the intestines of 5 pikes ranged from 48 to 52 cm were used. After dissecting the intestine and extracting the chyme and the worms, the mucosa was removed with a plastic spatula, homogenized and diluted with Ringer's solution in the mass-volume ratio of 1:49. All homogenates were prepared using glass homogenizers made by Sartorius AG (Germany).

The worm and mucosa homogenates were centrifuged at 6500 × g for 5 min at 4 °C. The washing samples, worm incubation medium and homogenates of worms and mucosa were frozen for further use.

The protein content in the incubation medium and worm homogenate was determined using Lowry's method (Lowry *et al.*, 1951).

Measuring the proteolytic activity

The total activity of alkaline proteinases in the tegumental washing samples and in the homogenate of the pike intestine mucosa was determined by the modified method of Alarcón using 0.3 % (w/v) azocasein in Tris buffer, pH 7.5 as substrate (Alarcón *et al.*, 2002). While preparing the control samples, equivalent volume of buffer solution was added to each sample instead of enzymatically active components. The substrate and enzymatically active sample were incubated for 60 min at 20 – 22 °C. The reaction was stopped by adding 1 ml of 0.3 M trichloroacetic acid (TCA) solution. The sediment of non-hydrolyzed protein was removed by centrifuging at 6500 × g for 5 min. The color development proportional to enzymatic activities was measured in the supernatant at 440 nm using the Lambda 25 (PerkinElmer) spectrophotometer.

Measuring the inhibitor activity

When measuring the inhibitor activity of worms, the proteinase sources were the homogenate of pike intestine mucosa and commercial trypsin from porcine pancreas (MP Biomedicals, USA; activity 250 USP). Trypsin was used in concentrations 0.0025, 0.005, 0.0075, 0.01 and 1 mg/ml in Tris buffer (pH 7.5). To determine the inhibitor activity, 50 – 150 µl of worm homogenate or incubation medium were added to the experimental medium containing 0.5 ml of mucosa homogenate or trypsin solution of certain concentration, and incubated for 15 min. Simultaneously, the same volume of buffer was added to the respective control sample. After the incubation, the proteolytic activity in the samples was determined as described above.

To identify the proteinase subclasses in washing samples F_1 – F_3 obtained from the tegument of *T. nodulosus*, 50 µl of the following

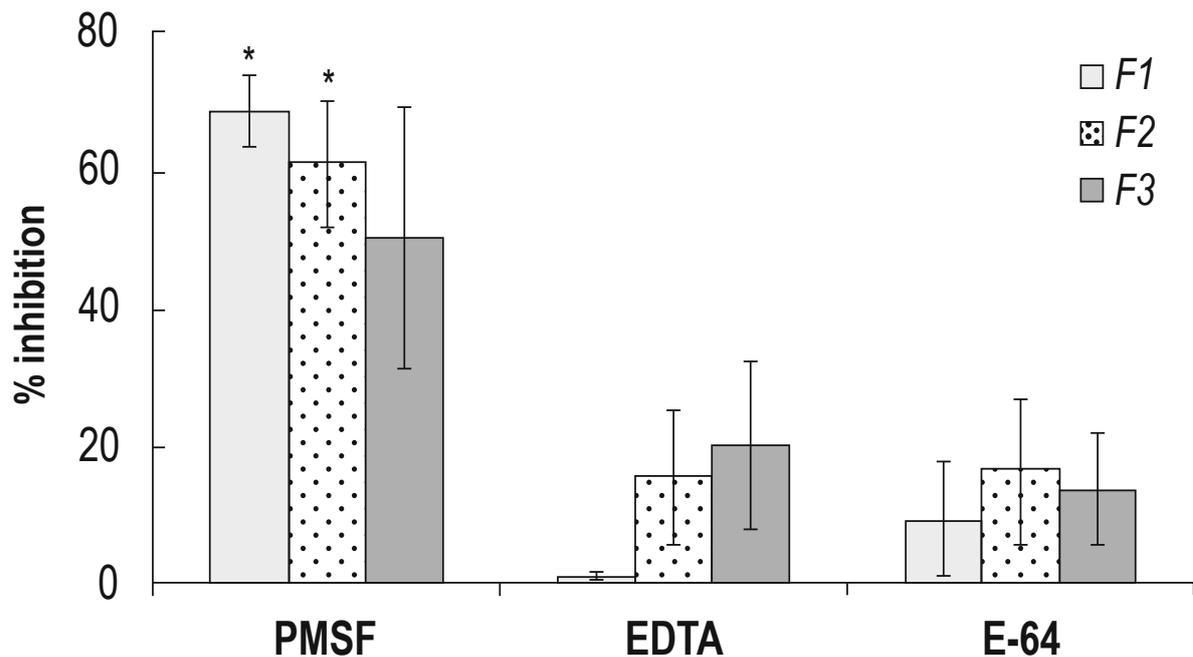


Fig. 1. Percent inhibition (mean \pm SE) of proteolytic activity by various inhibitors in different fractions (F_1 - F_3) washed off *T. nodulosus* tegument (*significant inhibitory effect, $p < 0.05$).

Inhibitors (50 μ l of each) were added to each test sample: PMSF – inhibitor of serine proteinases (100 mM solution in DMSO), EDTA – metalloprotease inhibitor (0.5 M in 1 M NaOH), and E-64 – inhibitor of cysteine (thiol) proteinases (1 mM in distilled water). Equal volumes (50 μ l) of Tris buffer (pH 7.5) were added to each control sample instead of inhibitor solution (100% inhibition means total suppression of enzyme activity). Fractions were washed off the worms by shaking them in 10 ml of Ringer's solution: for the first 30 seconds (F_1), for the next 15 minutes in fresh Ringer's solution (F_2), and for the final 15 minutes in a new portion of Ringer's solution (F_3). Seven randomly selected groups of worms were tested ($n = 7$).

inhibitors were used: (1) PMSF (phenyl-methyl-sulfonyl-fluoride) – inhibitor of serine proteinases, 100 mM solution in DMSO (dimethyl sulfoxide); (2) EDTA (ethylenediaminetetraacetic acid) – metalloprotease inhibitor, 0.5 M in 1 M NaOH, and (3) E-64 – inhibitor of cysteine (thiol) proteinases, 1 mM in distilled water.

Besides, to compare the inhibitor activity levels of *T. nodulosus* samples, the PMSF inhibitor activity in the pike intestine mucosa homogenate and trypsin solutions was measured.

The proteinase activity was expressed as the difference of spectrophotometer readings (Δ Abs) for the sample with substrate versus a blank sample per gram of wet worm, intestinal tissue or trypsin solution for a minute interval (Δ Abs/g/min). Each biochemical assay was conducted in triplicate.

Statistical analyses

The results are given as means \pm SE. Inhibitory effects were tested using a one-way ANOVA with a post hoc Dunnett's test when comparing multiple means against a single control mean or with the Tukey's (HSD) test for multiple pairwise comparisons. The level of significant difference was set at $p = 0.05$. Statistical analyses were carried out using the software package STATISTICA 6.0 (StatSoft, Inc., Tulsa, OK, USA).

Results

Proteolytic activity in tegumental washing samples

The research results show that proteolytic enzymes are washed off the worm's surface mainly to fraction F_1 , where the enzyme activity reached $0.49 \pm 0.03 \Delta$ Abs/g/min. In fractions F_2 and F_3 , only low-level proteolytic activity has been detected (0.003 ± 0.001 and $0.002 \pm 0.001 \Delta$ Abs/g/min respectively). It was demonstrated that PMSF notably reduces the activity of proteinases washed off the tegument. In fraction F_1 , PMSF inhibits $68.9 \pm 5.0\%$ of the activity (Fig. 1). On the contrary, no significant influence of EDTA and E-64 on the activity of desorbed proteinases was observed. In fractions F_2 and F_3 , no significant effect of any of the studied inhibitors on proteinase activity was detected (Fig. 1).

The pH value and protein content in the studied samples

It was ascertained that after 24 hours of worm incubation the pH value in Ringer's solution decreases from 7.5 to 6.4 ± 0.1 . At the same time, protein appeared in the incubation medium, and its content averaged 0.57 ± 0.16 mg/ml. The protein content in the homogenate of the studied cestodes reached 18.19 ± 1.99 mg/ml.

Inactivation of proteolytic enzymes by *T. nodulosus*

It was found that both the incubation medium and the worm homogenate reduce the proteolytic activity in the intestinal mucosa

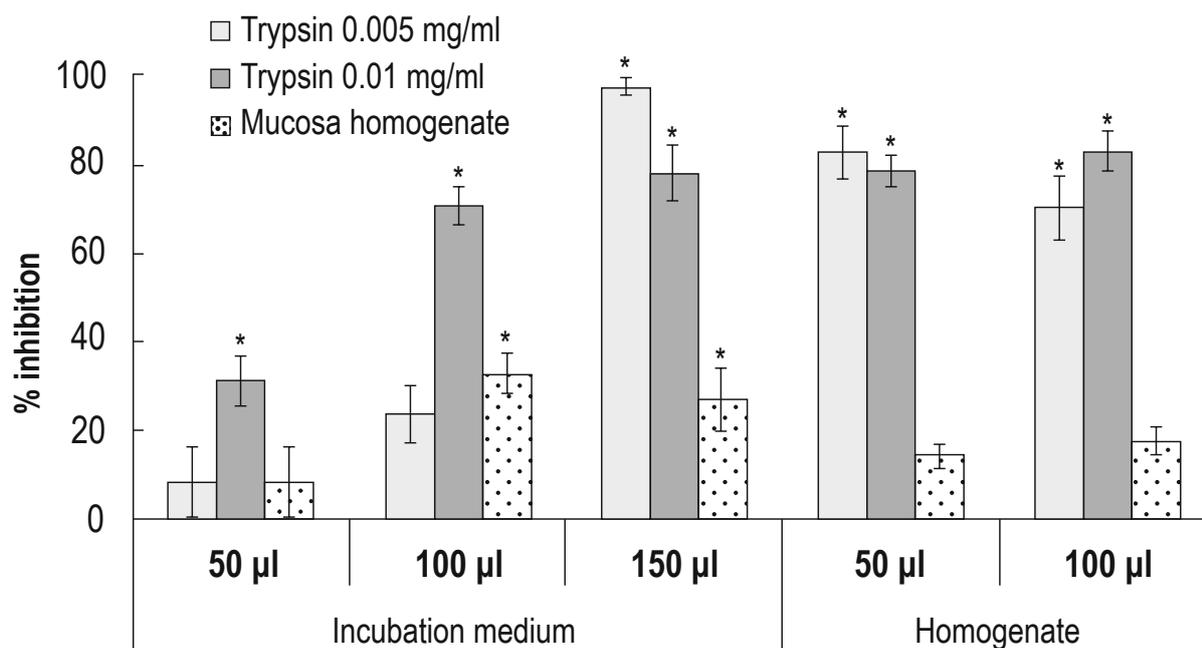


Fig. 2. Inhibitory action of *T. nodulosus* incubation medium and worm homogenate depending on sample volume (mean \pm SE; *significant inhibition, $p < 0.05$). The proteinase sources were commercial trypsin sample (MP Biomedicals, USA; activity 250 USP) with concentrations of 0.005 and 0.01 mg/ml in Tris buffer (pH 7.5) and the homogenates of pike intestinal mucosa. The mucosa was homogenized, diluted with Ringer's solution in the ratio of 1:49 and then centrifuged at 6500 \times g for 5 min at 4 $^{\circ}$ C. Incubation medium is 10 ml of Ringer's solution where the worms were kept for 24 hours at a temperature of 10 $^{\circ}$ C. The worm homogenates were diluted with Ringer's solution in the mass-volume ratio of 1:9 and centrifuged under the same conditions. In the experiments, 50 or 100 μ l volumes of supernatant were used. Control is the activity of a certain trypsin solution (or mucosa homogenate) with 50, 100 or 150 μ l of Tris buffer (pH 7.5) added instead of the same volumes of enzymatically active components (100% inhibition means total suppression of enzyme activity). Numbers of groups of worms tested were 5 for the incubation medium ($n = 5$) and 11 for the homogenate ($n = 11$).

homogenate and the activity of trypsin at various concentrations as compared to the control (Fig. 2). The extent and significance of activity reduction depend on the source of proteolytic enzymes (homogenate or trypsin) and the studied sample (worm incubation medium or worm homogenate) (Fig. 2).

The comparison of percent inhibition for trypsin solution and intestinal mucosa homogenate shows that both the worm incubation medium and its homogenate have a stronger inhibitory effect on the trypsin activity than on the mucosa homogenate. The trypsin inhibition percentage varied from 23.9 ± 6.1 % to 97.7 ± 1.9 % depending on its concentration, as well on the source and volume of the inhibitor. Conversely, suppression of the proteolytic activity in the mucosa homogenate ranged from 14.4 ± 2.8 just to 33.0 ± 4.6 %, and the inhibitory effects were significant only for some of the studied variants (Fig. 2).

The influence of various inhibitor sources on the activity of trypsin at a concentration of 0.01 mg/ml is shown in Fig. 3. Various volumes of the incubation medium and tapeworm homogenate significantly reduced the trypsin activity at this concentration. The efficacy of the inhibitors produced by the worm is comparable to that of the synthetic inhibitor PMSF. Besides, the detected effects were not dependent on the volume of the studied sample (Fig. 3).

When adding 50 μ l of worm homogenate to trypsin solutions of various concentrations, it was shown that the trypsin inhibition percentage depends on its concentration. A high degree of inhibition was found for trypsin concentrations of 0.005 – 0.01 mg/ml. The observed effects are significant, $p < 0.05$ (Fig. 4). At concentration of 0.0025 mg/ml, the trypsin activity reduction was not significant, and at concentration of 1 mg/ml no inhibition of the enzyme activity by the worm homogenate was seen (Fig. 4). It should be noted that the activity of trypsin at concentration of 0.01 mg/ml was slightly higher than at 0.005 mg/ml, but this difference was not significant ($p = 0.414$). The inhibitory action of 50 μ l of homogenate tested at trypsin concentrations of 0.005 – 0.01 mg/ml varied within a narrow range (from 78.6 ± 3.0 to 83.8 ± 7.2 %) amounting to approximately 80 %.

Discussion

Proteolytic activity in tegumental washing samples

The results of studying the desorption of proteinases from the tegument of *T. nodulosus* agree with the earlier obtained data on desorption of hydrolytic enzymes from the cestode tegument: the major activity is represented by the easily desorbed fraction

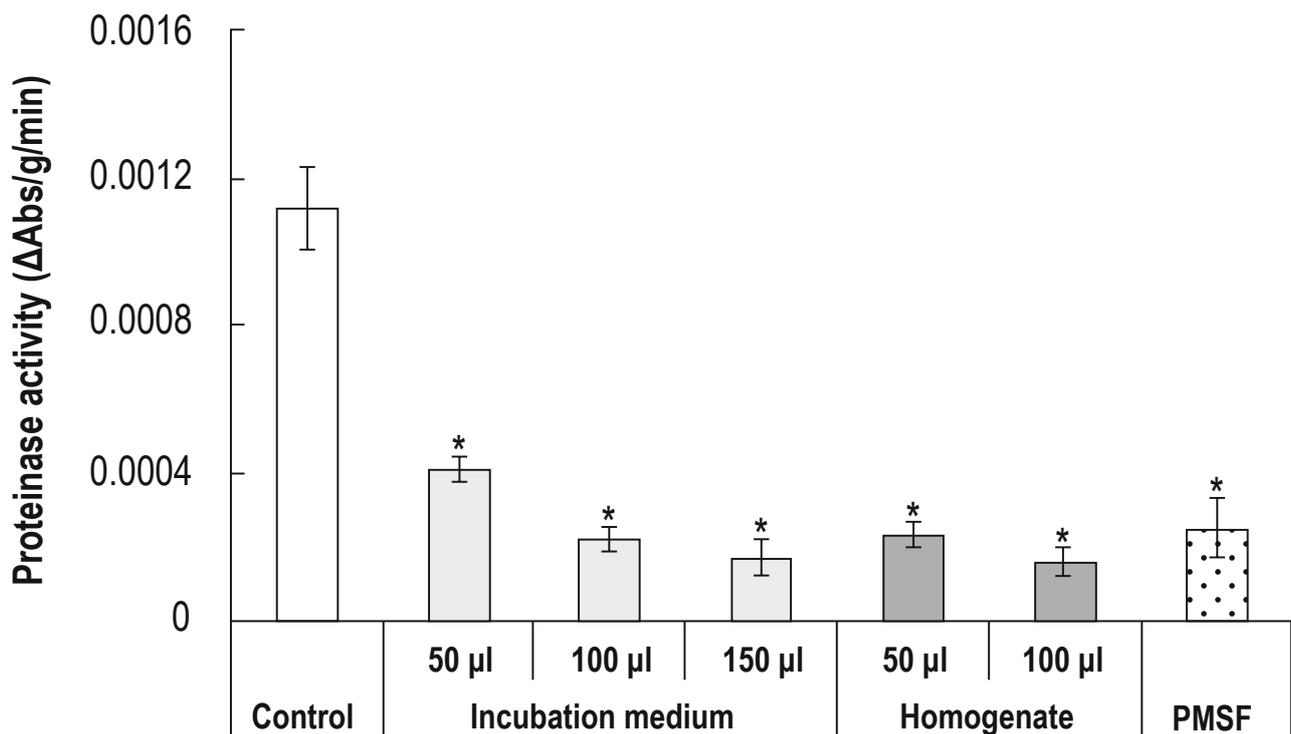


Fig. 3. Inhibitory effect of various volumes of *T. nodulosus* incubation medium, worm homogenate and PMSF on the activity of commercial trypsin samples (0.01 mg/ml) (mean \pm SE; *significant difference from a single control mean, $p < 0.05$).

The proteinase activity is the difference of spectrophotometer readings (Δ Abs) for the sample with substrate versus a blank sample per gram of trypsin solution for a minute interval (Δ Abs/g/min). Control is the activity of commercial trypsin sample (0.01 mg/ml). PMSF is the inhibitor of serine proteinases (50 μ l per each sample, 100 mM solution in DMSO). Incubation medium is 10 ml of Ringer's solution, where the worms were kept for 24 hours at a temperature of 10°C. The worm homogenate was diluted with Ringer's solution in the mass-volume ratio of 1:9 and centrifuged at 6500 \times g for 5 min at 4 °C. In the experiments, 50 or 100 μ l volumes of supernatant were used. Numbers of groups of worms tested were 5 for the incubation medium ($n = 5$) and 11 for the homogenate ($n = 11$).

characterizing the activity of the host's enzymes (Izvekova *et al.*, 1997). It is known that cestodes not only produce their own hydrolases but also capture and adsorb the host's enzymes, including proteinases (Halton, 1997; Izvekova *et al.*, 1997; Kuz'mina *et al.*, 2000; Dalton *et al.*, 2004; Izvekova, 2006). The inhibitor analysis shows that the main part of enzymes adsorbed on the tegument are serine proteinases. In the pike intestine, the highest percentage of proteolytic activity is also associated with serine proteinases and, according to some data, is 56.4 and 59.6 % in pikes uninfected and infected by *T. nodulosus* respectively (Izvekova & Solovyev, 2016).

Inactivation of proteolytic enzymes by cestodes

The obtained data show that *T. nodulosus* cestodes produce substances suppressing the activity of proteinases found in the host intestine mucosa homogenate, as well as the activity of trypsin solutions of different concentrations. According to some authors, enzyme inactivation is a marker of inhibitor production and secretion that requires no proof of the actual inhibitor presence (Matská-

si & Juhász, 1977). In our study, the comparable levels of trypsin activity reduction by the synthetic inhibitor PMSF and inhibitors produced by the worm show that the cestode's inhibitors inactivate serine proteinases (Fig. 3). This agrees with the data obtained by other authors showing the production of serine proteinase inhibitors by cestodes (Pappas, 1978; Pappas & Uglem, 1990). Serine proteinase inhibitors are among the key components of secretory products in many parasite species (Zimic *et al.*, 2007). They play an important role in the parasite's survival because of their ability to inhibit the host's enzymes, and are normally present in the microenvironment and/or secreted by the immune effector cells (Zang & Maizels, 2001). These inhibitors regulate the protease activity and control various processes related to it. Particularly, they play an important role in protecting the parasite from the host's digestive enzymes and, according to some authors, contribute to host specificity (Hawley & Peanasky, 1992). In the current study we established that *T. nodulosus* preparations had greater inhibitory effect on the trypsin activity than on the proteolytic activity of pike mucosa homogenate (Fig. 2). This is probably due to the fact

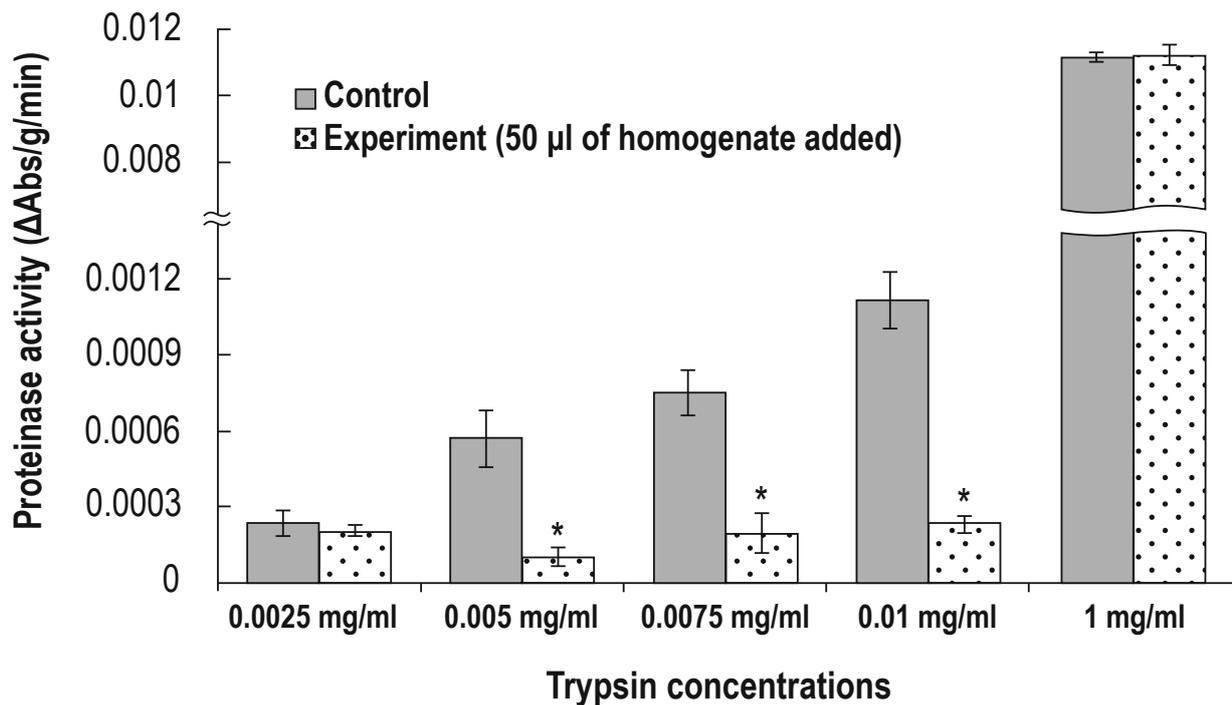


Fig. 4. Suppressive effect of *T. nodulosus* homogenate (50 μ l) on trypsin activity at various concentrations. Error bars indicate SE (*significant difference from respective control values, $p < 0.05$).

The proteinase activity is the difference of spectrophotometer readings (Δ Abs) for the sample with substrate versus a blank sample per gram of trypsin solution for a minute interval (Δ Abs/g/min). Control is the activity of commercial trypsin sample at various concentrations (0.0025 – 1 mg/ml) with 50 μ l of Tris buffer (pH 7.5) added to the reaction mixture. In the experiment, the activity of commercial trypsin sample in a certain concentration was determined with 50 μ l of worm homogenate added. The worm homogenate was diluted with Ringer's solution in the mass-volume ratio of 1:9 and centrifuged at 6500 \times g for 5 min at 4 $^{\circ}$ C. In the tests, 50 μ l of supernatant was used. Numbers of groups of worms tested was 11 ($n = 11$).

that the proteinase activity in the intestine is provided not only by trypsin, but also by chymotrypsin and various peptidases (Solovyev *et al.*, 2015). The study of the inhibitory capacity of *H. diminuta* also showed incomplete inhibition of the host's proteolytic activity by the worms, and great variability of the obtained data (Pappas & Uglem, 1990).

The detected effect of proteinase inhibition by the worm's incubation medium as well as by its homogenate means that the parasite spontaneously excretes the inhibitor into the medium (Fig. 2, 3). However, the increase in the volume of incubation medium and homogenate does not lead to significant changes in the proteinase inactivation level (Fig. 3). The present study ascertained that after 24 hours protein is detected in the *T. nodulosus* incubation medium. This may be associated with the regular renewal of the glycocalyx (Oaks & Lumsden, 1971), as well as with secretion of proteinase inhibitor into the surrounding medium. It is also known that proteinase inhibitors in many parasitic worms are of protein nature (Knox, 2007).

The insignificant influence of cestode homogenate on trypsin activity at its very low (0.0025 mg/ml) and considerably high (1 mg/ml) concentrations (Fig. 4) may be due to the insufficient sensitivity of the method in the former case, and to "masking" the inhibition effect at high concentration and, respectively, high activity in the

latter case. A reduction of the inhibitory activity as the enzyme concentration grows is also observed in *H. diminuta* (Pappas & Read, 1972a; Pappas, 1978).

Our experiment conditions (keeping the worms in Ringer's solution to get an incubation medium, and subsequent preparation of the worm homogenate) make it possible to suppose that secretion of proteinase inhibitors is not induced by the presence of proteinases in the medium, but instead, they are spontaneously synthesized by the parasite. A similar assumption was made earlier when exploring the soluble fraction of the isolated *H. diminuta* tegument brush border (Pappas & Uglem, 1990). The authors demonstrated that this fraction contains substances inhibiting the proteolytic and amidase activity of bovine trypsin and some proteolytic enzymes of the host's small intestine (Pappas & Uglem, 1990).

Possible mechanisms of proteinase inhibition in cestodes: facts and hypotheses

It should be noted that the available information on cestodes inhibiting proteolytic enzymes is somewhat contradictory. Thus, there are data confirming the inactivation of proteases during interaction with live worms. It was shown that the adsorptive surface of worms interacts with the host's enzymes altering their activity. For instance, during incubation of *H. diminuta* with trypsin, α - and

β -chymotrypsin *in vitro*, the specified enzymes were inactivated on the worm's surface, but no secretion of the active inhibitor into the surrounding medium was detected (Pappas & Read, 1972a,b). According to these authors, inactivation of trypsin and chymotrypsin was more dependent on the worm's weight than on its surface area. This may be associated with the renewal of glycocalyx that occurs in *H. diminuta* in 6 hours (Oaks & Lumsden, 1971). Later it was evidenced that trypsin is more likely adsorbed on the worm's surface than absorbed by the worm, however, the enzyme adsorption is not associated with its inactivation (Schroeder & Pappas, 1980; Schroeder *et al.*, 1981).

At the same time it was found that the isolated membrane of the *H. diminuta* tegument is not resistant to the action of proteolytic enzymes and does not affect their activity. Therefore it was assumed that inactivation of proteolytic enzymes requires a metabolically active, dynamic membrane, while an isolated tegument membrane sample does not meet these requirements (Pappas, 1987). Our findings of proteinase inhibition by the incubation medium and cestode homogenate show that inhibitors are secreted by worms. The isolation of tegument brush border is possibly accompanied by inhibitor loss at the stage of centrifuging used to obtain the samples. When studying the inhibitory capacity of *H. diminuta* inhabiting the rat intestine, it was found that salt solution with initial pH values from 5.5 to 10.0 is acidified by the worms to pH 5.0 (Uglen & Just, 1983). It was supposed that intestinal parasites may protect themselves from being digested by host enzymes by regulating the ambient pH, or by producing trypsin inhibitors, or both (Uglen & Just, 1983). However, we cannot fully agree with this supposition. In particular, for fish it was found that the activity of alkaline proteinases is displayed within the pH range from 5 to 10 (Solovyev *et al.*, 2015), while during the incubation of *T. nodulosus* in Ringer's solution the pH values decrease to 6.4 ± 0.1 . The reduction of pH values in *T. nodulosus* incubation medium is mainly related to secretion of lactic acid into this medium (Izvekova, 2001). Like most endoparasites, when having access to unlimited food resources, cestodes use shortcut metabolic pathways and perform incomplete food substrate oxidation (Soprunov, 1987). As a result, they produce and secrete a lot of partly oxygenated short-chain organic acids (lactic, propionic, acetic) lowering the host intestine pH. This suppresses the host intestine transport functions, while the worm's glucose carriers work better at lower pH values (Halton, 1997; Dalton *et al.*, 2004). Thus, parasites reach high efficiency and competitiveness of transportation systems, while pH reduction in the intestine does not cause a significant negative effect on the functioning of the host's digestive enzymes.

Although the available information, including our data, is somewhat contradictory, it is obvious that cestodes inhabiting the intestine of their definitive hosts are able to suppress the activity of host proteinases. The secretion of host enzyme inhibitors by parasites proves that the inhibitors play an important role in the parasite's life. Parasites produce inhibitors to survive and not be hydrolyzed by the host's proteinases. The dualism of helminth adaptation to the

living conditions is manifested in the interaction with the host's enzymes on the micro-level: the parasite uses the enzymes to hydrolyze the protein components of food on the one hand, and to inhibit proteinases on the other hand. The obtained data supplement the view of *T. nodulosus* functioning at the adult worm stage and its relationship with the definitive host.

Thus, the study shows that the main proteolytic activity in washings off the *T. nodulosus* tegument is represented in the easily desorbed fraction most likely characterizing the host enzyme activity. The activity of this fraction is mainly related to serine proteinases. At the same time, it is shown that *T. nodulosus* cestodes can inhibit host proteinases and commercial trypsin samples. Both the worm incubation medium and their homogenate have inhibitory activity. Obviously, proteinase inhibitor secretion is not induced by the presence of proteinases in the external medium, but spontaneously performed by the parasite. The inhibitor produced by the cestode appears to be trypsin-specific.

Acknowledgements

This work was supported by the Russian Foundation for Basic Research (grant number 15-04-02474).

References

- ALARCÓN, F.J., MARTÍNEZ, T.F., BARRANCO, P., CABELLO, T., DÍAZ, M., MOYANO, F.J. (2002): Digestive proteases during development of larvae of red palm weevil, *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Curculionidae). *Insect Biochem. Mol. Biol.*, 32: 265 – 274. DOI: 10.1016/S0965-1748(01)00087-X
- DALTON, J.P., SKELLY, P., HALTON, D.W. (2004): Role of the tegument and gut in nutrient uptake by parasitic platyhelminths. *Can. J. Zool.*, 82: 221 – 232. DOI: 10.1139/z03-213
- DELANEY, A., WILLIAMSON, A., BRAND, A., ASHCOM, J., VARGHESE, G., GOUD, G.N., HAWDON, J.M. (2005): Cloning and characterisation of an aspartyl protease inhibitor (API-1) from *Ancylostoma* hookworms. *Int. J. Parasitol.*, 35: 303 – 313. DOI: 10.1016/j.ijpara.2004.11.014
- DUBOVSKAYA, A.YA. (1973): A study of proteolytic activity in some cestode species. *Parazitologiya*, 7: 154 – 159 (In Russian)
- DZIK, J.M. (2006): Molecules released by helminth parasites involved in host colonization. *Acta Biochim. Pol.*, 53(1): 33 – 64
- HALTON, D.W. (1997): Nutritional adaptations to parasitism within the Platyhelminthes. *Int. J. Parasitol.*, 27: 693 – 704. DOI: 10.1016/S0020-7519(97)00011-8
- HAWLEY, J.H., PEANASKY, A.J. (1992): *Ascaris suum*: Are trypsin inhibitors involved in species specificity of ascarid nematodes? *Exp. Parasitol.*, 75: 112 – 118
- HWANG, J.H., LEE, W.G., NA, B.K. *et al.* (2009): Identification and characterization of a serine protease inhibitor of *Paragonimus westermani*. *Parasitol. Res.*, 104: 495 – 501. DOI: 10.1007/s00436-008-1219-6

- IZVEKOVA, G.I. (2006): The nutritional adaptations of lower cestodes – fish parasites. *Usp. Sovrem. Biol.*, 126: 630 – 642 (In Russian)
- IZVEKOVA, G.I. (2001): *Trienophorus nodulosus* (Cestoda, Pseudophyllidea): final stages of carbohydrate metabolism. *Helminthologia*, 38: 23 – 27
- IZVEKOVA, G.I., KUPERMAN, B.I., KUZ'MINA, V.V. (1997): Digestion and digestive-transport surfaces in cestodes and their fish host. *Comp. Biochem. Physiol. A.*, 118: 1165 – 1171. DOI: 10.1016/S0300-9629(97)00040-6
- IZVEKOVA, G.I., SOLOVYEV, M.M. (2016): Characteristics of the effect of cestodes parasitizing the fish intestine on the activity of the host proteinases. *Biol. Bull.*, 43: 146 – 151. DOI: 10.1134/S1062359016010076
- KAGEYAMA, T. (1998): Molecular cloning, expression and characterization of an *Ascaris* inhibitor for pepsin and cathepsin E. *Eur. J. Biochem.*, 253: 804 – 809. DOI: 10.1046/j.1432-1327.1998.2530804.x
- KLIMENKO, V., KĚNIŇA, V. (1971): On the influence of phylogeny and ecology on some biochemical mechanisms of helminth adaptation. *Latvijas Zinatnu akadēmijas vestis*, 11: 93 – 96. (In Russian)
- KNOX, D.P. (2007): Proteinase inhibitors and helminth parasite infection. *Parasite Immunol.*, 29: 57 – 71. DOI: 10.1111/j.1365-3024.2006.00913.x
- KUZ'MINA, V.V., IZVEKOVA, G.I., KUPERMAN, B.I. (2000): Peculiarities of nutrition physiology in cestodes and their host-fish. *Usp. Sovrem. Biol.*, 120: 384 – 384. (In Russian)
- LI, A.H., MOON, S.-U., PARK, Y.-K. *et al.* (2006): Identification and characterization of a cathepsin L-like cysteine protease from *Taenia solium* metacestode. *Vet. Parasitol.*, 141: 251 – 259. DOI: 10.1016/j.vetpar.2006.05.015
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L., RANDALL, R.J. (1951): Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193: 265 – 275
- MATSKÁSI, I. (1984): The effect of *Bothriocephalus acheilognati* infection on the protease and α -amylase activity in the gut of carp fry. *Symp. Biol. Hung.*, 23: 119 – 125
- MATSKÁSI, I., JUHÁSZ, S. (1977): *Ligula intestinalis* (L., 1758): Investigation on plerocercoids and adults for protease and protease inhibitor activities. *Parasitol. Hung.*, 10: 51 – 60
- MOLEHIN, A.J., GOBERT, G.N., MCMANUS, D.P. (2012): Serine protease inhibitors of parasitic helminths. *Parasitology*, 139: 681 – 695. DOI: 10.1017/S0031182011002435
- OAKS, J., LUMSDEN, R.D. (1971): Cytochemical studies on the absorptive surfaces of cestodes. V. Incorporation of carbohydrate containing macromolecules into tegument membranes. *J. Parasitol.*, 57: 1256 – 1268
- PAPPAS, P.W. (1987): *Hymenolepis diminuta*: interactions of the isolated brush border membrane with proteolytic enzymes. *Exp. Parasitol.*, 64: 38 – 47
- PAPPAS, P.W. (1978): Tryptic and protease activities in the normal and *Hymenolepis diminuta*-infected rat small intestine. *J. Parasitol.*, 64: 562 – 564
- PAPPAS, P.W., READ, C.P. (1972a): Trypsin inactivation by intact *Hymenolepis diminuta*. *J. Parasitol.*, 58: 864 – 871
- PAPPAS, P.W., READ, C.P. (1972b): Inactivation of α - and β -chymotrypsin by intact *Hymenolepis diminuta* (Cestoda). *Biol. Bull.*, 143: 605 – 616
- PAPPAS, P.W., UGLEM, G.L. (1990): *Hymenolepis diminuta* (Cestoda) liberates an inhibitor of proteolytic enzymes during in vitro incubation. *Parasitology*, 101: 455 – 464
- RASCÓN, A.A.JR, MCKERROW, J.H. (2013): Synthetic and natural protease inhibitors provide insights into parasite development, virulence and pathogenesis. *Curr. Med. Chem.*, 20: 3078 – 3102. DOI: 10.2174/0929867311320250005
- REICHENBACH-KLINKE H.-H., REICHENBACH-KLINKE K.-E. (1970): Enzymuntersuchungen an Fishen. II. Trypsin- und α -amylase-Inhibitoren. *Arch. Fishereiwiss.*, 21: 72 – 76
- SCHROEDER, L.L., PAPPAS, P.W. (1980): Trypsin adsorption by *Hymenolepis diminuta* (Cestoda). *J. Parasitol.*, 66: 49 – 52
- SCHROEDER, L.L., PAPPAS, P.W., MEANS, G.E. (1981): Trypsin inactivation by intact *Hymenolepis diminuta* (Cestoda): some characteristics of the inactivated enzyme. *J. Parasitol.*, 67: 378 – 385
- SHISHOVA-KASATOKHINA, O.A., LEUTSKAYA, Z.K. (1979): *Biochemical aspects of relations between helminth and host*. Moscow, Nauka, 280 pp. (In Russian)
- SOLOVYEV, M.M., KASHINSKAYA, E.N., IZVEKOVA, G.I., GLUPOV, V.V. (2015): pH values and activity of digestive enzymes in the gastrointestinal tract of fish in lake Chany (West Siberia). *J. Ichthyol.*, 55: 251 – 258. DOI: 10.1134/S0032945215010208
- SOPRUNOV, F.F. (1987): *Molecular basis of parasitism*. Moscow, Nauka, 224 pp. (In Russian)
- UGLEM, G.L., JUST, J.J. (1983): Trypsin inhibition by tapeworms: antienzyme secretion or pH adjustment? *Science*, 220: 79 – 81.
- ZANG, X., MAIZELS, R.M. (2001): Serine proteinase inhibitors from nematodes and the arms race between host and pathogen. *Trends Biochem. Sci.*, 26: 191 – 197. DOI: 10.1016/S0968-0004(00)01761-8
- ZIMIC, M.J., INFANTES, J., LÓPEZ, C. *et al.* (2007): Comparison of the peptidase activity in the oncosphere excretory/secretory products of *Taenia solium* and *Taenia saginata*. *J. Parasitol.*, 93: 727 – 734. DOI: 10.1645/GE-959R.1