Abstract

Monogeneans from the family Diplozoidae (subclass Heteronchoinea) are bloodfeeding ectoparasites inhabiting gills of common carp. Digestion of blood in diplozoids is an intracellular process taking place in gut cells within lysosomal cycle in the presence of parasite's peptidases. However, information about the blood digestion comes only from ultrastructural and histochemical analyses. Therefore, I have focused in this work on biochemical and molecular characteristics of bioactive molecules which may participate in blood processing by *E. nipponicum* adults, especially cysteine peptidases of cathepsin L- and B- types, aspartic peptidases of cathepsin D-type, and Kunitz-type inhibitors of serine peptidases.

In homogenates and excretory/secretory (E/S) products of *E. nipponicum* adults, an activity of cysteine peptidases of cathepsins L-type dominated, followed by an activity of cathepsin D-like aspartic peptidases and a minor cathepsin B-like activity. Inhibitors of the abovementioned peptidase types completely blocked hemoglobinolytic activity in the samples. In the transcriptome of *E. nipponicum* adults, ten cathepsin L-coding transcripts were found and only one cathepsin B-coding transcript. Primary structures of the encoded enzymes were bioinformatically and phylogenetically compared. Two abundant cathepsins L (EnCL1, EnCL3) were expressed in *P. pastoris*/*E. coli* expression systems and then biochemically and functionally characterized. Both enzymes were localized inside the gut cells and also in gut lumen of the adult worm. The results suggested probable extracellular phase of blood digestion in *E. nipponicum* and also the presence of a wide range of cathepsins L with different structural characteristics and also with likely different functions.

In addition to peptidases participating in the degradation of blood proteins, haematophagous parasites also need bioactive molecules inhibiting coagulation during the blood intake. To regulate the haemostasis, they often use inhibitors of serine peptidases which participate in the coagulation cascade. These include, e.g., Kunitz-type inhibitors which were also found in the transcriptome of adult *E. nipponicum*. One of them (named EnKT1) was chosen for heterologous expression in *E. coli* system. Recombinant EnKT1 inhibited peptidolytic activity of human factor Xa, plasmin and plasma kallikrein *in vitro*. Its anticoagulation activity based on inhibition of factor Xa was confirmed *in vitro* by tromboelastography. Despite the ability to inhibit plasmin, its impact on fibrinolysis was not confirmed. On the other hand, EnKT1 effectively inhibited cytolytic activity of fish

complement *in vitro*. By means of *in situ* hybridization and immunohistochemistry, the EnKT1 was localized inside the digestive cells of the parasite. It possesses a secretory signal sequence and was detected in E/S products of the worms. Based on these characteristics, we suppose that EnKT1 serves as a bifunctional inhibitor dininishing coagulation of ingested blood in the parasite's gut and, simultaneously, protecting cells of the gastrodermis from complement attack. It is the first example of a parasite-originated Kunitz protein with confirmed inhibitory effect on complement cascade in host’s blood.