

Abstract

For the successful food intake by organisms that feed on blood is essential presence of antihemostatic molecules such as vasodilators, anticoagulant molecules and apyrases. Although members of family Diplozoidae (Heteronchoinea) are blood-feeding parasites on the gills of the fish, these molecules, that could disrupt host hemostasis, have not yet been identified.

Thus, the aim of this study was to find molecules with potential anticoagulant activity in homogenates of whole worm bodies and excretory/secretory products of the members of family Diplozoidae. Furthermore perform bioinformatics analysis of sequences obtained from transcriptome project of *Eudiplozoon nipponicum* (Heteronchoinea: Diplozoidae) and selected proteins (protein domain) then expressed in a recombinant form.

We tested inhibitory activity in excretory-secretory products and homogenates of members family Diplozoidae towards coagulation factors IIa and Xa and their specific fluorogenic with 4 negative and 1 positive results.

From the results of two transcriptome analysis we discovered three protein families of potential anticoagulants - annexins, serpins and Kunitz-domain proteins. For further analyses we focused on the Kunitz protein family. These proteins contain one or more structurally related active domains which are able to inhibit the function of proteases. By production of selected recombinant Kunitz proteins and their domains in *Escherichia coli* and *Pichia pastoris* we intend to confirm their anticoagulation activity by inhibition tests towards coagulation factors. The recombinant Kunitz domain wasn't expressed.

Key words: coagulation cascade, hemostasis, anticoagulant, thrombin, factor Xa, blood, haematophagy, parasite, Diplozoon, Eudiplozoon, Paradiplozoon

