

Abstract

This study focuses on the recombinant cysteine peptidases – cathepsin B originating in the bird schistosome *Trichobilharzia regenti* that is unique across the whole family for its ability to migrate through the nerve tissue to the final localization. For invasion, migration, degradation of nutritional proteins and/or evasion of host immune responses, schistosome employs peptidases.

This study follows the research done by researchers of Department of parasitology, Faculty of Natural Sciences, Charles University. The main goal of this study was to deepen the characteristics of recombinant cathepsins B originating in *T. regenti*.

In *T. regenti*, two cysteine peptidases – cathepsins B1 (TrCB1) and B2 (TrCB2) – have been previously characterized. TrCB1 is located in the gut of schistosomula and involved in digestion. TrCB2 occurs in post-acetabular penetration glands of cercariae and probably facilitates penetration.

The recombinant pro-cathepsin B (isoforms TrCB1.1, TrCB1.4 and also TrCB2) were expressed in *Pichia pastoris* yeast system. An attempt was made to produce in *P. pastoris* the recombinant isoform TrCB1.6, in which the active site cysteine is substituted by glycine.

While TrCB2 underwent self-processing in the expression medium, TrCB1.1 and TrCB1.4 zymogens were effectively activated only after the incubation with Prep-High-S Support (a matrix for ion exchange chromatography) or with pepsin. With the use of specific synthetic substrate Hippuryl-His-Leu-OH the exopeptidase activity was confirmed in the case of recombinant cathepsins B. All 3 recombinant cathepsins B were able to degraded selected protein substrates – myelin basic protein, fibrinogen, serum albumin, IgG, collagen and partially hemoglobin. Subsequently, using MALDI-MS/MS, LC-MS/MS the cleavage sites were identified in the substrates of myelin basic protein and hemoglobin which resulted from the activity of these enzymes.

Keywords: cathepsin B, *Trichobilharzia*, Schistosoma, peptidase, recombinant protein, enzym, *Pichia*