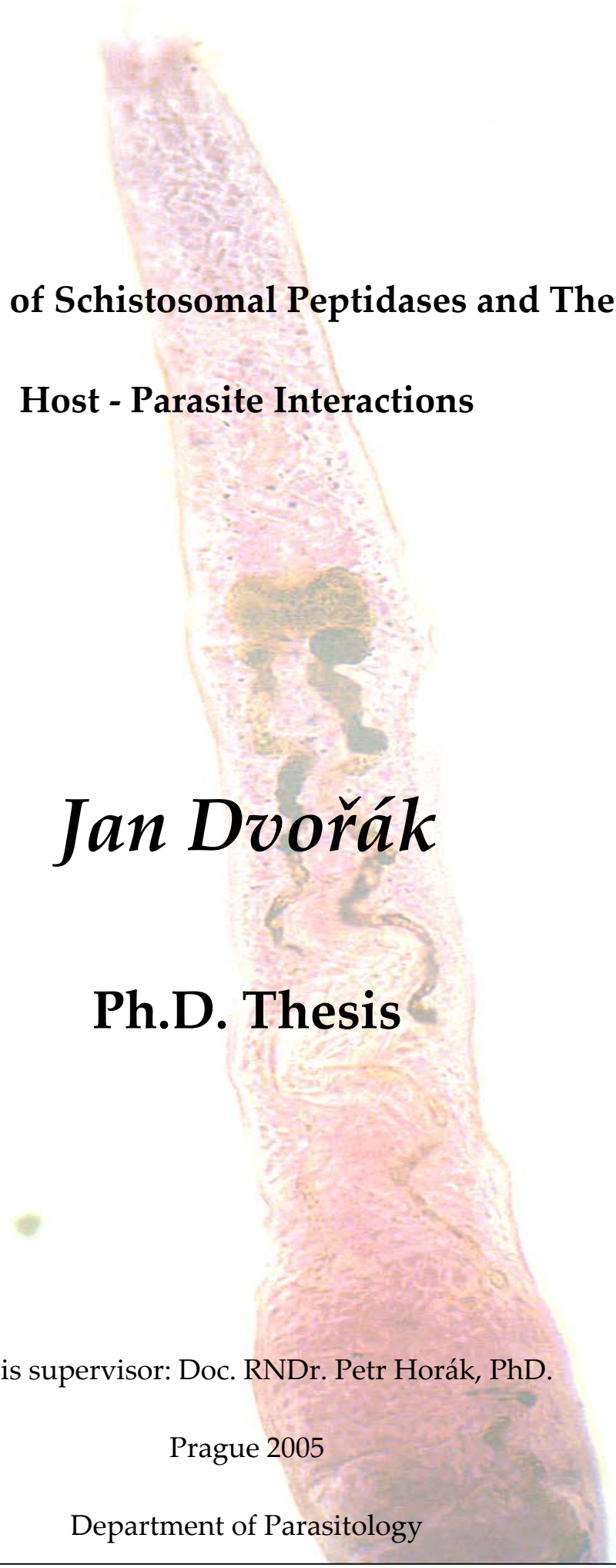

Characterization of Schistosomal Peptidases and Their Role in Host - Parasite Interactions

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Ph.D. Thesis

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Introduction

Peptidases are important enzymes for host-parasite interactions and they are critical for successful parasite survival. In schistosomes, various peptidases play roles in parasite metabolism, migration and immunoevasion. Up to date, research was mostly focused on peptidases from human schistosomes. Their enzymes have been described and characterized as promising vaccine and drug targets. Bird schistosomes from the genus *Trichobilharzia* were neglected. Their free-swimming invasive larvae (cercariae) are able to penetrate the skin of nonspecific mammalian hosts (including humans) and can cause cercarial dermatitis also known as "swimmers' itch". They are also able, as transformed larvae (schistosomula), to survive for a limited period in mammalian tissues. Data about peptidases of *Trichobilharzia* participating during the host skin invasion and migration are fractional and insufficient.

Moreover, the bird nasal schistosome *Trichobilharzia regenti* has been described as a neuropathogen. Schistosomula of *T. regenti* migrate through the peripheral and central nervous system to the nasal area. Their migration can cause neuromotor disorders and paralysis of bird hosts (ducks) and also experimentally infected nonspecific mammalian hosts (mice). The nervous tissue serves as a source of nutrition during migration and development, whereas, in the nasal tissue, adult flukes feed on blood. To date, there was no information regarding peptidases elaborated by *T. regenti* that might facilitate digestion of or migration through the nervous tissue.

In the PhD thesis, most of the experimental work has been focused on peptidases from the neuropathogenic *T. regenti*, and partly also on peptidases from *Trichobilharzia szidati* and *Schistosoma mansoni*.

Aims of the thesis

1. Characterization of peptidases from neuropathogenic *Trichobilharzia regenti* schistosomula (principal topic)

a) Development of methods for isolation of worms from the nervous tissue.
b) Detection of proteolytic activities in worm homogenates.
c) Purification of predominant peptidases and/or alternatively to directly employ molecular methods in order to obtain sequence information.
d) Cloning and expression of selected peptidases in appropriate expression system in order to characterize their biochemical properties and biological roles.
e) Characterization of activation mechanisms *in vitro* of heterologously expressed enzymes.
f) Using immunochemistry and natural protein substrates, partial characterization of recombinant peptidases with respect to their function *in vivo*.

2. Detection and characterization of cysteine peptidases from *T. regenti* and *T. szidati* cercariae (additional topic with participation as a team member)

3. Comparative study of the inhibitory effects of selected aza-asparagine Michael acceptors on *Schistosoma mansoni* asparaginyl endopeptidase (SmAE aka *S. mansoni* legumain)

Thesis conclusions

1. Peptidases from migratory neuropathogenic *Trichobilharzia regenti* schistosomula

Worms were isolated using 150 μm pore wire mesh in Tris-buffered saline or RPMI-1640 medium. Using fluorogenic synthetic peptidyl substrates, specific inhibitors and irreversible affinity probes; a predominant 33-34 kDa cysteine peptidase was detected and tentatively identified as an ortholog of *Schistosoma mansoni* cathepsin B1. Molecular methods were directly employed for detailed studies. Degenerate primers designed upon the sequence consensus of active proteolytic sites of other schistosomal cathepsin B enzymes were used for PCR analysis. Six isoforms of cathepsin B in *T. regenti* schistosomula (termed TrCB1.1 – 1.6.) were detected. Isoforms TrCB1.5 and TrCB1.6 were identified as inactive enzyme homologs. Two proteolytically active isoforms (TrCB1.1 and 1.4) were successfully expressed in *Pichia pastoris*. Both activated isoforms exhibited different specificity and pH profiles for cleaving peptidyl substrates. TrCB1.1 zymogen was unable to undergo auto-activation, but it was *trans*-processed and activated by *S. mansoni* asparaginyl endopeptidase (SmAE). TrCB1.4 zymogen activated itself at low pH and SmAE was unable to facilitate its activation. Specific polyclonal antibodies localized peptidases exclusively in the gut of schistosomula and reacted with the previously detected 33 kDa protein responsible for cathepsin B-like activity in schistosomula extracts. Both isoforms degraded myelin basic protein, the major protein component of the nervous tissue, but were inefficient against mammalian and bird hemoglobins.

2. Detection of cysteine peptidases from *T. regenti* and *T. szidati* cercariae

Specific irreversible affinity probe identified 33 and 31 kDa clan CA cysteine peptidases in *T. regenti* and *T. szidati* cercariae, respectively. Inhibitors and peptidyl substrates identified a predominant cathepsin B-like activity in cercarial extracts and E/S products.

3. Inhibitory studies of *Schistosoma mansoni* asparaginyl endopeptidase (SmAE).

Several aza-asparagine Michael acceptors were proved as potent inhibitors of SmAE with IC50 values in the low nanomolar range (IC50 = 31-55 nM). These inhibitors could be useful for studies of the role of SmAE in schistosomal metabolism.