University of South Bohemia
Faculty of Biological Sciences
Department of Molecular Biology and Biochemistry

**Rigorózní práce**

# IrAE: an asparaginyl endopeptidase (legumain) in the gut of the hard tick *Ixodes ricinus*

**Mgr. Daniel Sojka**

**Supervisor: RNDr. Petr Kopáček, CSc.**

Institute of Parasitology,
Biology Centre, Academy of Sciences of the Czech Republic
Laboratory of Vector Immunology

**České Budějovice, 2007**

**Sojka D., 2007:** IrAE: an asparaginyl endopeptidase (legumain) in the gut of the hard tick *Ixodes ricinus*. Rigorózní práce, v anglickém jazyce, 13 stran. Jihočeská univerzita v Českých Budějovicích, Biologická fakulta, České Budějovice.

**Annotation:**
This work is focused on the newly described cysteine protease - the legumain-like protease referred as *Ixodes ricinus* asparaginyl endopeptidase (IrAE). It was shown to be expressed solely in the gut and to be localized inside the gut digestive cells and within the peritrophic matrix. The enzyme was fully cloned, and the active recombinant prepared in the yeast *P. pastoris* was characterized using specific substrates, inhibitors and visualization with activity based probes. IrAE was shown to digest hemoglobin directly in vitro in a discrete manner, but the overall hemoglobinolytic activity is rather weak. We suppose that IrAE resembles its schistosomal orthologue as a major processive enzyme activating other protease precursors in a network or cascade of cysteine and aspartic proteases that likely perform the major portion of blood protein digestion in ticks.

**Financial Support:** This work was supported by grants to P.K.: No. A6022307 from the Grant Agency of the Academy of Sciences of the Czech Republic; No. 206/06/0865 from the Grant Agency of the Czech Republic and the Research Centre No. LC06009. We thank the Sandler Family Supporting Foundation for financial support of research visit of D.S. to the San Francisco laboratory. D.S. was also supported by project FRG3/3646/2005 and PhD program No. 524/03/H133 from the Ministry of Education, Youth and Sports of the Czech Republic. Research at the Institute of Parasitology, BC ASCR and the Faculty of Biological Sciences, USB is covered by research plans Z60220518 and MSMT 6007665801, respectively.

*I hereby declare that all the work summarized in this thesis was done on my own or in collaboration with co-authors of the presented papers and manuscript and only using the cited literature and personal communication.*

Mgr. Daniel Sojka

České Budějovice, 2007

**Abstract**

Ticks are ectoparasitic blood-feeders and important vectors for pathogens including arboviruses, rickettsiae, spirochetes and protozoa. As obligate blood-feeders, one possible strategy to retard disease transmission is disruption of the parasite's ability to digest host proteins. However, the constituent peptidases in the parasite gut and their potential interplay in the digestion of the blood meal are poorly understood.We have characterised a novel asparaginyl endopeptidase (legumain) from the hard tick Ixodes ricinus (termed IrAE), which we believe is the first such characterisation of a clan CD family C13 cysteine peptidase (protease) in arthropods. By RT-PCR of different tissues, IrAE mRNA was only expressed in the tick gut. Indirect immunofluorescence and EM localised IrAE in the digestive vesicles of gut cells and within the peritrophic matrix. IrAE was functionally expressed in Pichia pastoris and reacted with a specific peptidyl fluorogenic substrate, and acyloxymethyl ketone and aza-asparagine Michael acceptor inhibitors. IrAE activity was unstable at pH P 6.0 and was shown to have a strict specificity for asparagine at P1 using a positional scanning synthetic combinatorial library. The enzyme hydrolyzed protein substrates with a pH optimum of 4.5, consistent with the pH of gut cell digestive vesicles. Thus, IrAE cleaved the major protein of the blood meal, hemoglobin, to a predominant peptide of 4 kDa. Also, IrAE trans-processed and activated the zymogen form of Schistosoma mansoni cathepsin B1 – an enzyme contributing to hemoglobin digestion in the gut of that bloodfluke. The possible functions of IrAE in the gut digestive processes of I. ricinus are compared with those suggested for other hematophagous parasites. 