Recombinant cysteine peptidases of Ixodes ricinus

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Abstract

Intracellular proteolysis of ingested blood proteins is a crucial physiological process in ticks. In a tick, *Ixodes ricinus,* cysteine proteases, cathepsins B and L, are part of a gut-associated multi-peptidase complex. This thesis deals with preparation of recombinant procathepsins B and L and characterization of functional and biochemical properties of cathepsins B and L. After expression procathepsins B and L in *E. coli* and their purification from inclusion bodies by affinity chromatography, the basic strategy was searched that would enable to achieve successful refolding. A suitable type in both cases showed to be ”basic” refolding. Both refolded procathepsins were auto-activated in the acidic environment of pH 4,0; cathepsin B (but not cathepsin L) also at pH 5,5. Activity was determined at pH dependence substrate specificity and inhibition. The pH optimums for hydrolytic activity of cathepsins B and L were in the range of 4,5 – 5,5 and 3,0 – 3,5, respectively. Both enzymes were blocked by an inhibitor of cysteine peptidase group and each of them by specific inhibitors designed for mammalian cathepsins B and L. The results of this thesis show that cathepsins B and L produced in the gut *I. ricinus* exhibit exo- and endo-peptidase activity, which confirms their supposed role in the cascade of proteolytic degradation of proteins from the blood of the host. These proteins are potential antigens for development of a vaccine to prevent sucking of ticks and transmission of pathogens.