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

Rigorózní práce

Lysozyme from the gut of the soft tick *Ornithodoros moubata*: the sequence, phylogeny and post-feeding regulation.

Lenka Grunclová

Supervisor: RNDr. Petr Kopáček, CSc.
Biology Centre of the Academy of Sciences of the Czech Republic, v.v.i.
Institute of Parasitology
Laboratory of Vector Immunology

České Budějovice, 2007

Grunclová L., 2007: Lysozyme from the gut of the soft tick *Ornithodoros moubata*: the sequence, phylogeny and post-feeding regulation. Rigorózní práce, in English – 10p., Faculty of Biological Sciences, University of South Bohemia, České Budějovice.

Annotation: Sequence of a tick gut lysozyme (TGL) from the soft tick *Ornithodoros moubata* was determined by cloning and sequencing of overlapping polymerase chain reaction (PCR) and RACE PCR products. The phylogenetic analysis revealed that the TGL belongs to the c-type lysozymes, to an H-branch due to a unique histidine residue at position 52. TGL seems to be a case in which the features of lysozymes with anti-bacterial and digestive function are combined. Semiquantitative RT-PCR and Northern blotting analysis demonstrated that TGL is strongly up-regulated at the transcriptional level after a blood meal. It is the first lysozyme sequence representing the subphylum Chelicerata.

I declare that this thesis was performed by me or in collaboration with co-authors and with the help of cited literature.

České Budějovice, May 9, 2007 Lenka Grunclová

Lysozyme from the gut of the soft tick *Ornithodoros moubata*: the sequence, phylogeny and post-feeding regulation

Lenka Grunclová, Hélène Fouquier, Václav Hypša, Petr Kopáček

Dev. Comp. Immunol. 2003. 27: 651–660.

Abstract:

Sequence of a tick gut lysozyme (TGL) from the soft tick *Ornithodoros moubata* was determined by cloning and sequencing of overlapping polymerase chain reaction (PCR) and RACE PCR products. It is the first lysozyme sequence representing the subphylum Chelicerata. The resulting open reading frame codes for a putative signal peptide of 22 amino-acid residues and a mature protein composed of 124 amino-acids. Calculated mass of the protein is 14037.75 Da and a theoretical isoelectric point is 8.16. The phylogenetic analysis revealed that the TGL belongs to the c-type lysozymes. It forms a distinct monophyletic group together with multiple lysozyme-like sequences found in the gene products agCP6542 from *Anopheles gambiae* strain PEST and CG8492-PA from *Drosophila melanogaster*. This group is referred to as an H-branch due to a unique histidine residue at position 52 which replaces the highly conserved tyrosine present in the vast majority of c-type lysozymes. TGL seems to be an interesting case in which the features of lysozymes with anti-bacterial and digestive function are combined. Semiquantitative RT-PCR and Northern blotting analysis demonstrated that TGL is strongly up-regulated at the transcriptional level after a blood meal. The maximum lysozyme mRNA level was detected 16 h post blood meal and the message remained stable for 5 days and then it slowly dropped down to the level of non-fed ticks within 2 weeks.