

Paitoon Kaewhom 2009: Molecular Cloning and Expression of Serpin Gene from Salivary Glands of *Rhipicephalus microplus*. Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor Sathaporn Jittapalapong, Ph.D. 74 pages.

Tick salivary gland proteins have potential for application as novel tick control agents. The serine protease inhibitors secreted from salivary glands of the tick may be used in an anti-tick feeding vaccination. In the present study, the recombinant serpin (rserpin) protein of cattle tick's salivary glands was expressed and determines the immunogenicity of rserpin in rabbits. Serpin cDNA was cloned from the *Rhipicephalus microplus* salivary glands by RT-PCR and analyzed its nucleotide and deduced amino acid sequence. The results showed that the 1,200 bp open reading frame of serpin could encode a protein with 399 amino acid residues. By comparison with other serpins available in the GenBank database, the amino-acid sequence in the reactive center loop (RCL) of the cloned serpin showed a 95% and 100% identity to those of the *Rhipicephalus microplus* and *Rhipicephalus appendiculatus*, respectively.

The rserpin protein expressed in *Pichia pastoris* was used to immunize rabbits 3 times at 2 weeks interval at 100 µg rserpin per rabbit. SDS-PAGE analysis of crude supernatant has shown a distinct band of approximately 45 kDa. Western blot analysis also gave a specific band approximately the same size as that of SDS-PAGE. By ELISA, all immunized rabbits generated antibodies against rserpin at the first week of immunization and reached its peak within the seventh week. The antibody titer was analyzed by ANOVA and showed significantly differences between the control groups (group 1: PBS and group 2: WT protein) and the immunized group from the third week of immunization through the end of the experiment. This result indicated that rserpin had the strong immunogenicity and might be the candidate antigen for the anti-tick vaccine. However, it is necessary to perform a clinical trail with target animals as natural hosts.

Student's signature

Thesis Advisor's signature

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