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Molecular cloning, expression, purification and characterization of 37 kDa serine protease activation in pupal midgut of silkworm, *Bombyx mori*

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Silkworm *Bombyx mori* is an important economic insect for the production of silk. After the silkworm genome project was completed, silkworm has been explored as a model organism for understanding basic physiology and biochemistry of insects. In insects, protease is playing an essential role in digestion of food and physiological process including, apoptosis and tissue remodelling. The 2D electrophoresis results confirmed that the p37k serine protease was synthesized as a zymogenic precursor during the larval stage in the gut of lumen, secret into the epithelium and then activated upon pupation. It is also proposed that 37 kDa serine protease is responsible for apoptosis during pupal- adult transformation. The p37k activation and its further role in B.mori are not well understood. Hence the present study, p37k serine protease zymogen gene was characterized and sequenced. It shows an open reading frame of 987-bp encoding a 329aa. Then it was cloned and expressed in*Escherichia coli* strain BL21 (DE3) using pET 30a expression vector. The SDS-PAGE analysis revealed thatexpression of desired protein at 42.5 kDa upon IPTG induction (0.5mM). Purified his-tagged enzyme gave a single band with a molecular mass of 42.5 kDa on SDS-PAGE and confirmed by MALDI-TOF-MS analysis. The protease activity of p37k was observed through zymography after treatment with a commercial enzyme (Furin) and early pupalmidgut extract. This suggest that activation of p37k in the pupal stage of the process of pro-protein conversion of zymogen to mature p37k through arginine site specific cleavage (RT/RRRI) enzyme (Furin like enzyme) in the pupal stage. Likewise, several enzymes have been synthesized as a zymogen in different stage and activated time-specific manner in many insect pests for its growth and development. Identification of zymogen (pro-protein) processing enzyme and design of an inhibitor carrying anti-apoptotic character will be used as a drug in future.

Biography

M Krishnan has completed his PhD in the year 1985 from Madurai Kamaraj University and joined as a lecturer in the year 1989 in Bharathidasan University then he was elevated as a Chair, Professor and Head in the Department of Environmental Biotechnology, Bharathidasan University. He has published more than 100 papers in well reputed journals. He has enhanced his skills by bringing fund from various funding agencies also from foreign funds for the improvement of University and department. He has produced 20 PhD students.

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