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RESEARCH ARTICLE **OPEN ACCESS**

MOLECULAR ENDOCRINE MODEL MECHANISM OF INSECT METAMORPHOSIS AND JH ACID AS THE KEY REGULATOR

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ABSTRACT

Metamorphosis comprise dramatic transformation in shape and function of organs, tissues and individual cells. According to the classical theory of the hormonal control of insect metamorphosis, ecdysteroids initiates a molt independent on the titer of JH. However a few observations earlier indicate that tissues must first acquire competence in the presence of JH acid alone is not sufficient for the metamorphic response to ecdysteroid. JHacid is an inactive precursor and metabolite of JH actually induces cells to become competent to undergo metamorphoses, whereas ecdysteroid merely stabilizes this commitment and facilitates the expression of this state of development program.The model system used in this project is the common Mormon butterfly Papillio polytes is a major pest of Rutaceous plants. Metamorphosis especially molting behavior in insects is known to be governed by specific dermal glands known as Version's glands. Ecdysteroid induces and coordinates the molting process and JH determines the nature of moult. JH acid is an inactive precursor and metabolite of juvenile hormone (JH) that induces cells to become competent to undego metamorphosis, whereas ecdysteroid merely stabilizes this commitment that facilitates the expression of this state of developmental programme.Verson's glands that are found specifically in lepidopteran insects are paired dermal glands of epidermal derivatives which contribute a protective layer to the newly formed cuticle or might has defensive function.In the present study localization of Version's glands were done The specific role of JH metabolite, the JH acid in the induction of metamorphic competence were examined. Elucidation of the fundamental mechanism and interaction of insect endocrine molecules during insect metamorphosis were also explained.

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INTRODUCTION

Metamorphosis comprise dramatic transformation in shape and function of organs, tissues and individual cells.Juvenile hormone (JH) was initially discovered in the 1930s as a factor that is secreted from the corpus allatum (CA) and inhibits insect metamorphosis. The chemical structure of the first JH was solved in 1967, and at least eight JHs have been identified to date. JH regulates development, reproduction, diapause, polyphenism,and behavior throughout insect life.JH biosynthesis is controlled by various neuroendocrine and neuronal factors in complex stage and species specific ways. JH has multiple functions, and a primary role of JH in insect development is to modulate ecdysone action. JH maintains the current commitment of the tissues and cells, whereas ecdysone causes both predifferentiative and differentiative cellular events that are necessary for the molt. Thus, when JH is present, a molt to a larval stage ensues. If JH is absent at the onset of the molt, metamorphosis occurs. According to the classical theory of the hormonal control of insect metamorphosis, ecdysteroids initiates a molt independent on the titer of JH.However a few observations earlier (Ismail *et al*., 2000) indicate that tissues must first acquire competence in the presence of JH acid alone is not sufficient for the metamorphic response to ecdysteroid. JHacid is an inactive precursor and metabolite of JH actually induces cells to become competent to undergo metamorphosis, where as ecdysteroid merely stabilizes this commitment and facilitates the expression of this state of development program. The model system used in this study is the comon mormon butterfly *Papilio polytes* is a major pest of Rutaceous plants.Its larval period lasts for 17-24 days thus completing its lifecycle within a period of conditions and seasonal variations. The 3rd, 4th and 5thinstars has an osmeterical gland in the first thoracic segment and it is defensive in function. The 5th instar larvae changes to prepupa before pupation on the plant itself as a naked C shaped chrysalis handling to the plant by spinning a silken girdle.The model part used in this study is versons glands which is found exclusively only in lepidopteran insects. Metamorphosis especially molting behaviour in insects is known to be governed by specific dermal glands known as Verson's glands. Ecdysteroid induces and coordinates the molting process and JH determines the nature of moult. These glands are paired dermal glands of epidermal derivatives which may contribute a protective layer to the new cuticle or may be defensive. Verson's gland was selected as the model system, because specific protein products from both larval and pupal stages can be made simultaneously by a cell in the midst of this

transition. In *papilio polytes* this gland is present as a pair on the anterodorsal region of each segment lying below the epidermis. Its size diminishes as it reaches the last abdominal segment and it is absent in last segment. Verson's glands show differences in protein patterns between a larval- pupal molt.

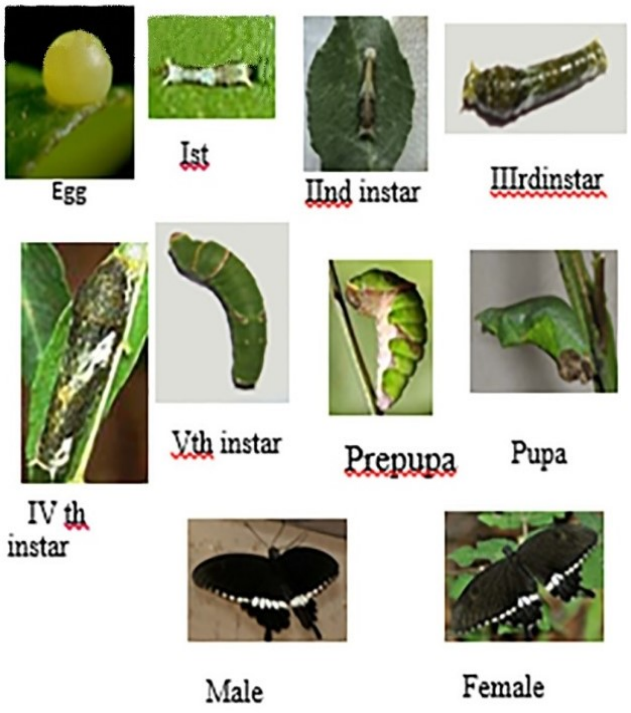
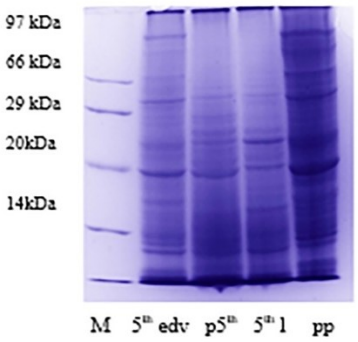


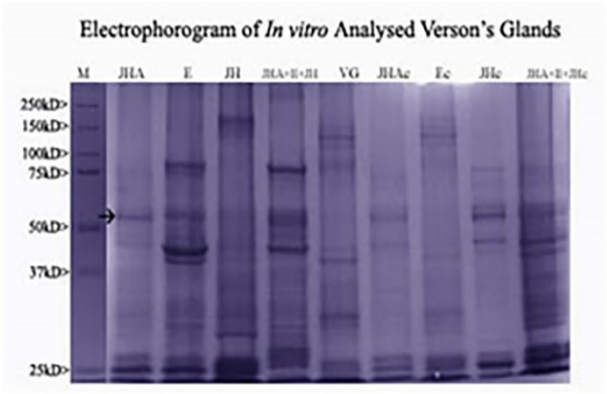
Figure 1. Life cycle pattern of *Papilio polytes*

MATERIALS AND METHODS

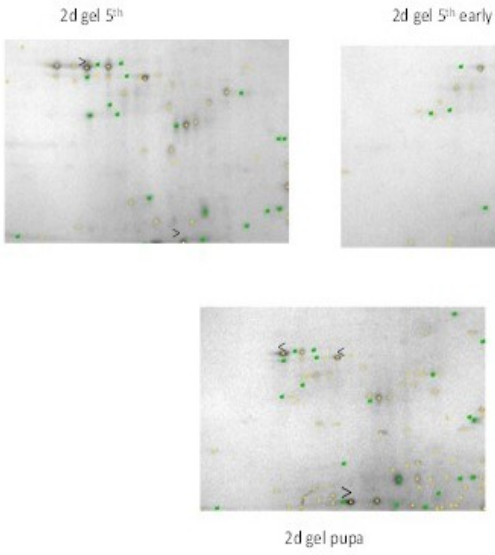
Localization of Verson's Glands is done by a square cuticle portion from muscle free segment was excised under microscope and adhering fat body and trachea were carefully cleared and the glands were collected. Dissected Verson's glands in MEM were homogenized in sample buffer, centrifuged at 14000 g for 5min, supernatant was collected and subjected to SDS-PAGE analysis on 10% SDS gels under constant voltage. 2DE were done by following parameters. Dissected out glands were homogenized in TRIS-DTT-PIC homogenizing medium, centrifuged at 14000 g for 5min, and supernatant was collected. First dimension was done using iso-electric focusing (IEF) at 500 V for 2 hrs, 1500 V for 1hr, 3500 V for 5 hrs.Second dimension was performed using SDS –PAGE (10%) under constant voltage. 2DE gels with the candidate protein spots were run and silver stained for MS analysis. Analysis was performed using an UltraFlex MALDI-TOF mass spectrometer. Spectra were analyzed using the Denovo software and calibrated internally with the auto-proteolysis peptides of trypsin.Blast analysis were done byl identifications based on one matching peptide or low Blast scores were manually verified, and all proteins that were identified only once were checked carefully. *In vitro* analysis were done with total of 90 Verson's glands were dissected out from the 5th instar of *Papilio poltytes* and kept in Grace's medium. 9 Verson's glands sets with with JH acid, Ecdysteroid and JH were incubated for 12h with 0.5µg/ml concentrations and 0.1µg/ml concentrations for another 12h .After incubation the glands were homogenized. The media and the glands were analysed separately by SDS-PAGE.

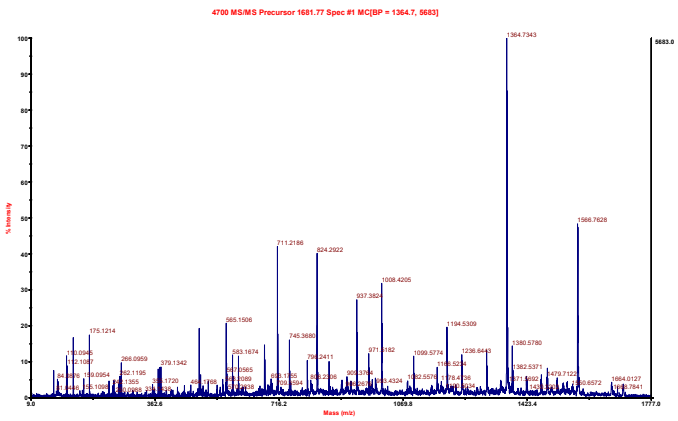
Secretory pattern and VGP profile during *P.polytes* developmental cycle



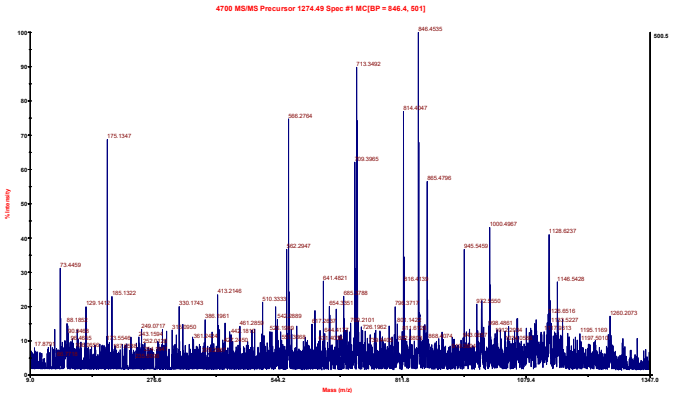


2 dimensional electrophorogram resolving VGP from *P. polytes*

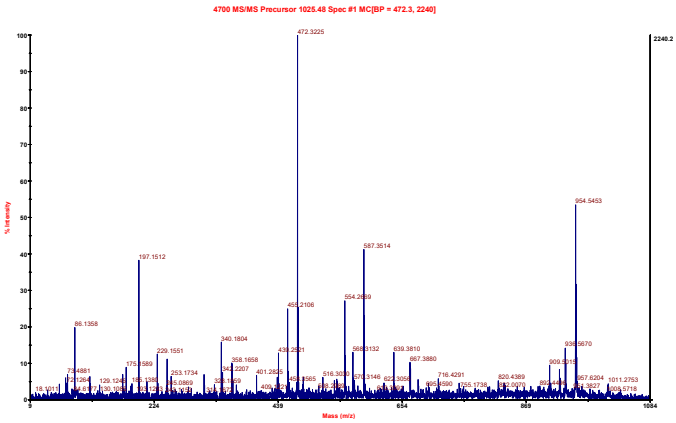




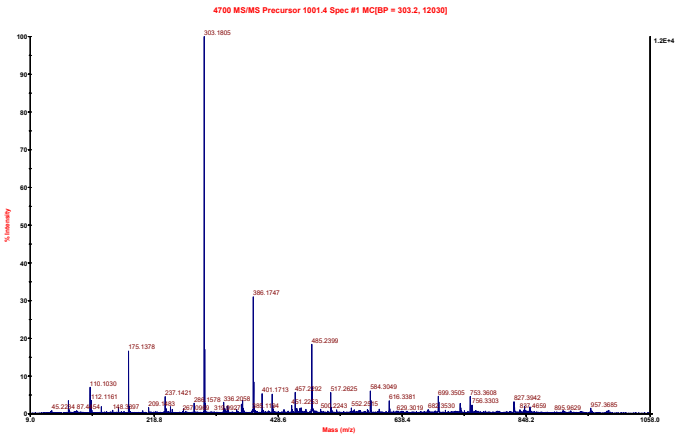
MS Analysed data of 5th1 early larval protein



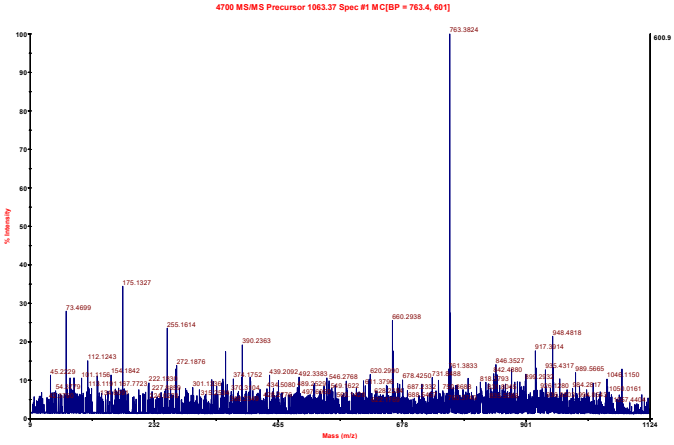
MS Analysed data of 5th 2 early larval protein



MS Analysed data of P1 larval protein



MS Analysed data of P2 larval proteins



MS Analysed data of P3 larval proteins

Life stage (*P.polytes*)	Peptide Sequence	Similarity	Function
5th inst 1	KSNVHMTSARK	Elongase [Culex]	Long chain fatty acid elongation
5th inst 2	VPIEDIIRA	unc79, isoform D [Drosophila]	protein, zinc ion binding
Pupal 1	MTHVVDGAR	serine hydroxymethyltransferase [Aedes]	Methane metabolism
Pupal 2	DGKCGGGAPCAK	CG15564-PA [Apis]	Predicted
Pupal 3	PSEVYLDLKF	Tissue plasminogen activator [PLAT]	serine protease [Plasminogen to plamin]

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RESULT AND DISCUSSION

*Papilio polytes*larval specific proteins resolved werelying within the molecular mass of 11-13 kDa andwhich are the primary larval secretary product. The pupal-specific proteins are first detected inlarvae with exposed dorsal vessel (EDV), an event that was triggered by ecdysteroid. After EDV 3 different polypeptides contributing to 30-34 kDa, 66 kDa and 75 kDa which are pupal-specific appears. Two dimensional electrophorograms confirm the stage-specific differences in larval and pupal proteins.The 2-DE analysed stage specific proteins – P1,P2,P3 , *5th 1 and 5th 2* of*Papilio polytes* were analysed by MS Analysis.Each of the stage specific trypsinated peptides usually produce different spectra.The peptides were analysed by denovo sequencing using the denovo software. The peptide sequences obtained after denovo sequencing were further ranked for BLAST analysis.BLATP algorithm to identify sequence similaritythe stage specific proteins of Verson's glands must be an unique protein. BLATP algorithm to identify sequence similarity the stage specific proteins of Verson's glands must be an unique protein.

CONCLUSION

The two different larval and pupal-specific protein units of same glandularorigin makes this an ideal marker for in depth morphogenetic hormonal action. This finding further provides information on an important component of insect cuticle and this novel information should definitely kindle more research interest in devising endocrine-based insect pest management strategy. Verson's Glands that secrete the cement layer of cuticular proteins appear to secrete stage specific – larval and pupal proteins in the holometabolan (*Papilio polytes*) seems to be under the control of JH acid level during different developmental stages.Studies on insect hormone are of both scientific and economic importance.From the scientific point of view,such studies are valuable for our understanding of the neuroendocrine process in insects and thereby introducing an interesting evolutionary aspect attempt to utilize JH endocrine system as a pesticide target to develop juvenoids or JH analogues. Common limitation of Juvenoids as pesticides is that they prolong the destructive instars of many pests and also only acting at specific periods of development.

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