tributed for reduced feeding and consequent reduction in larval population. Another interesting observation was the delayed pupation as a consequence of extended postembryonic development. By 69th day, only 22% of the larval population on Soluneem-treated palms pupated when compared to 38% in the untreated group. Such delayed post-embryonic development has been reported in several insects upon foliar application of neem-based formulations. A significant (P < 0.01) reduction in the average pupal weight was also observed in Soluneem treatment (Table 2) presumably due to reduced feeding on leaflets from Soluneem-treated palms. The most striking observation was the drastic reduction in adult eclosion as a consequence of the treatment (Table 1). A majority of the moths emerging from treated palms (66%) showed malformation, while all the control group pupae emerged into normal adults (Figure 1). An incidence of a fresh early instar larval population of the next generation was observed in both control and Soluneem-treated palms 110 days after initial systemic application (Table 1). However, a significantly (P < 0.01) lower number of larvae were recorded on leaflets of Soluneem-treated trees, which could be due to its extended protective effect.

Preliminary observations on the systemic application of Soluneem to coconut trees infested with the eriophyid mite, Aceria (Eriophyes) guerreronis have shown that it drastically reduces the population of these mites, which are spreading at an alarming rate in the southern parts of India threatening the coconut industry. In the light of the recent ban on the application of the synthetic pesticide, Monocrotrophos, Soluneem could be a safe alternative for the control of pests infesting not only coconut palms but also other economically important perennial trees by systemic application.


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Induction of subcellular malic dehydrogenase activity in fat body cells of diapausing pupae of wild tasar silkworm Antheraea mylitta Drury (Lepidoptera: Saturniidae) by 17-β estradiol

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For elucidation of vertebrate estrogen-induced responsiveness of fat body cells of insects, various doses (0.5, 1.0, 5.0, 10 and 50 μg) of 17-β estradiol (E2) were injected thrice, at 5 day intervals, to 130-day-old diapausing pupae of Antheraea mylitta and the changes in cytosolic NADP-dependent (cMDH, EC 1.1.1.40) and mitochondrial NAD+-dependent (mMDH, EC 1.1.1.37) malic enzyme activity together with protein content of the subcellular fractions was determined. NAD+-linked cMDH activity decreased in both the sexes at the doses of 10 and 50 μg of estrogen (E2) per pupa. Lower doses of E2 (0.5–5 μg) also showed a trend in reduction of specific activity of the enzyme although the data was not found to be significant statistically.

On the contrary, specific activity of NAD+-linked mMDH was enhanced with all the doses of estrogen used so far in both male and female insects. Protein content in tissue fractions of fat body cells of male and female pupae was elevated with all the doses of injected E2 when compared to control. In general, females contained more protein in both the tissue fractions than their male counterparts while in case of malic enzyme activity it was found to be just the reverse.

Hence, the activity of NAD+ cMDH and NAD+-mMDH in fat body cells and its responsiveness to the biologically active hormone estrogen in tasar silkworm has been documented as has been found in B. mori and other invertebrates. The steroid hormone effect in diapause regulatory mechanism is also established.

INACTIVATION of prothoracic glands (PgLs) leading to a deficiency of ecdysteroids is the main cause of pupal diapause in holometabolous insects1,2. The pupal brain stops releasing PTH in response to diapause programming signals, and hence inhibits the production of ecdysone from the PgLs, thereby interrupting the pupal-

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adult transformation and subsequent adult development which is manifested as pupal diapause. Bimodal generations of the wild tasar silkworm, Antheraea mylitta Drury, undergoes pupal diapause for an extended period of about 200-210 days depending on the ambient environmental conditions, although it remains to be clarified how this insect enters, maintains and terminates its diapause. Recently we have demonstrated that 150th day of pupal age in diapause destined A. mylitta is the critical phase for the initiation of diapause termination under normal circumstances. Application of vertebrate estrogen to diapausing pupae induced precocious adult emergence by advancing the reproductive maturation. Again, the octopaminergic neurons are physiologically responsive to 17β estradiol which is actively involved in diapause regulatory mechanism by triggering tissue metabolism in advance. Thus, we thought that vertebrate estrogen being a steroid hormone may exert its effect by mimicking 20 hydroxyecdysone (20E) when the Pgs remain inactive during diapause in A. mylitta. Sinha et al., reported that application of 20E prior to pupation in diapause destined generations of tasar silkworm breaks pupal diapause, thereby shortening the diapause duration to 25-52 days. Some literature is also available on the presence of vertebrate type sex steroid (estrogen) in silkworm Bombyx mori together with its metabolic significance. Very recently it has been found that the fat body, ovary and silk glands are the target organs for estrogen action in B. mori. In a previous study we have reported that plasma protein level increases on 150th day of diapausing pupa after administration of 17β estradiol besides altering the octopamine titre in A. mylitta and the hemolymph composition may be an indicator of the metabolic changes in some vital organs, specifically the fat body cells.

Considering the physiological importance of fat body cells, the present study has been undertaken to reveal the effect of estrogen on fat body tissue of A. mylitta specifically during diapause state. In this work we have chosen 'malate dehydrogenase' - the enzyme related to lipid metabolism since this enzyme system is well-known to be estrogen-sensitive in B. mori.

Healthy male and female diapausing pupae of wild tasar silkworm, A. mylitta were selected randomly from bimodal generations at the time of pupation (0 day pupae) and subjected to estrogen treatment under the same physiological conditions. Average body weight of the pupa was recorded to be 8.94 ± 0.20 g and 13.87 ± 0.38 g for male and females respectively. The pupa was maintained inside an insectary under ambient environmental conditions.

Estradiol 17β (Sigma) was dissolved in absolute alcohol:0.65% saline mixture (1:1). Three consecutive injections of E2 were given to the male and female diapausing pupae of tasar silkworm on 130, 135 and 140 days respectively at the doses of 0.5, 1.0, 5.0, 10.0 and 50.0 μg per pupa. The hormone injections were given with the help of 10 μl Hamilton microsyringe (Reno, Mv) through the intersegmental membrane between the 6th and 7th abdominal segment of the insect. The control animals received an equal volume of the vehicle (absolute alcohol: 0.65% saline mixture, 1:1). The injected volumes did not exceed 5 μl in any case. The insects were sacrificed on 150th day of pupal age for enzyme assay considering the fact that this is the critical phase for initiation of diapause termination in this insect.

Fat body tissue from the diapausing pupae were dissected out in cold and rinsed with ice cold 0.65% saline to make it free from body fluid. A measured amount of fat body tissue was homogenized in 1 ml of 0.25 M sucrose solution in a glass-teflon homogenizer (10 strokes per minute). The cell debris and nuclei were removed by centrifugation of the tissue homogenate in cold at 5000 g for 5 min. The supernatant was re-centrifuged at 16,000 g for 20 min at 4°C to obtain the mitochondrial pellet which was purified by washing twice with cold homogenizing medium. The final mitochondrial pellet was resuspended in 1 ml of the homogenizing medium, containing 100 μl of 0.05% Triton X-100 to disrupt the mitochondrial wall, for enzyme assay. The supernatant from the first 16,000 g centrifugation step was again respun at 105,000 g for 1 h to obtain the cytosolic fraction. The entire isolation and fractionation procedure of mitochondria and cytosol was conducted at 4°C.

NADP*-dependent cytosolic malic dehydrogenase (EC 1.1.1.40) was assayed following the method of Hsu and Lardy as modified by Murphy and Walker with certain modifications for insect fat body tissue of A. mylitta by using a reaction mixture containing 0.5 mM NADP*, 3.8 mM sodium L-malate, 0.05 mM MnCl2 and 48 mM triethanolamine (TEA) buffer at pH 7.4. The rate of NADP* reduction was measured at 340 nm for a period of 5 min using a Beckman Model-24 spectrophotometer. The activity of NAD*-dependent malate dehydrogenase (EC 1.1.1.37) was determined by the method of Englard and Siegei with slight modifications for insect tissue by using a reaction mixture containing 5 mM NAD*, 38 mM sodium L-malate and 16.12 mM glycine-NaOH buffer at pH 9.5. The rate of NAD reduction was measured at 340 nm for 5 min. The specific enzyme activity in both cases was expressed as ΔAbs/min/mg protein.

Protein content in fat body mitochondrial and cytosolic fractions was determined by the method of Lowry et al. and absorbance was read at 750 nm in a Shimadzu spectrophotometer. Bovine serum albumin (Sigma, USA) was used as protein standard. Data were subjected to statistical analysis using Student’s t test.

In this study, both NAD* and NADP*-linked malate enzyme activity has been demonstrated in fat body tissue...
sue of tasar silkworm A. mylitta, for the first time, as found in various tissues of other insects\textsuperscript{23-27}. In addition, estrogen-induced alterations in MDH activity and protein level in sub-cellular fractions of fat body cells of diapausing pupae of this insect has also been established. In general, NADP\textsuperscript{+}-dependent MDH activity in cytosolic fraction of fat body cells of male diapausing pupae of A. mylitta was recorded to be significantly higher ($P < 0.001$) (111.67\%) than its female counterparts (Figure 1). Exogenous application of estrogen at the doses of 10 and 50 $\mu$g/pupa caused a significant reduction ($P < 0.01$) in the enzyme activity in both the sexes. Lower doses (0.5–5 $\mu$g) of estrogen were unable to elicit significant changes in enzyme activity in fat body tissue, though the trend of reduction was noticeable in both the male and female silkworms.

In mitochondria, NADP\textsuperscript{+}-dependent MDH showed significantly higher ($P < 0.001$) activity in male (66.92\%) than the female pupa in a manner similar to the cytosolic fractions of fat body cells (Figure 2). Interestingly, vertebrate 17$\beta$ estradiol exerted its stimulatory effects on mitochondrial MDH unlike that in cytosolic mitochondrial MDH. In this case, all the doses of $E_2$ (0.5–50 $\mu$g/pupa) caused a significant enhancement ($P < 0.01$–$P < 0.001$) in NADP\textsuperscript{+}-linked MDH activity in both sexes in a dose-dependent manner. In female, application of exogenous estrogen at doses of 10–50 $\mu$g caused maximum increase in specific enzyme activity, while in case of males the maximum effective doses were recorded to be 5–10 $\mu$g.

NADP\textsuperscript{+}-linked malate dehydrogenase is directly involved with fatty acid chain elongation through the production of NADPH\textsuperscript{−} (ref. 28). According to Sacktor\textsuperscript{29}, it is reasonable to assume that the mitochondrial enzyme functions in tricarboxylic acid (TCA) cycle oxidations, whereas the cytoplasmic enzyme has a role in gluconeogenesis. On the other hand, Weeda and coworkers\textsuperscript{30} propose that NAD\textsuperscript{+}-linked malic enzyme may be involved in the generation of pyruvate during proline oxidation in the flight muscles of insects. The specific activity of NAD\textsuperscript{+}-linked malic enzyme in fat body cells mitochondria of A. mylitta is particularly high (93.77\%, irrespective of sex) as compared to NADP\textsuperscript{+}-linked cMDH. Our findings also corroborate the results of studies conducted by Das and Ray\textsuperscript{14} in the mulberry silkworm B. mori. NADP\textsuperscript{+}-linked malic enzyme have been the subject of several studies and have been reported in a variety of organisms, but usually with low activities\textsuperscript{31–34}. However, NAD\textsuperscript{+}-linked malic enzyme with high activity is found to predominate in insects\textsuperscript{30,35,36}.

Untreated control male diapausing pupae of tasar silkworm showed higher malic enzyme activity in cytosolic and mitochondrial fractions respectively compared to female sex. This may also be one of the important physiological causes for early eclosion of male moth\textsuperscript{b} in addition to octopamine titre in hemolymph\textsuperscript{b} which accelerates the rate of development during pupal–adult transformation. Such protandry fits the model of natural selection for optimum reproductive strategy for males\textsuperscript{37}. Reddy et al.\textsuperscript{38} have also documented this type of sexual dimorphism in other enzyme systems like ATPases in fat body cells of A. mylitta.

![Figure 1. Effect of estradiol-17β on malic enzyme activity (NADP\textsuperscript{+}-cMDH) in fat body cytosol of male and female pupae of A. mylitta. Three consecutive injections were given to 130, 135 and 140-day-old pupae. Vertical bars represent the standard error of the mean (ns, not significant; b, $P < 0.01$).](image-url)
Figure 2. Effect of estradiol-17β on malic enzyme activity (NAD+-mMDH) in mitochondrial fractions of fat body of male and female pupae of A. mylitta. Three consecutive injections were given to 130, 135 and 140-day-old pupae. Vertical bars represent the standard error of the mean (b, \(P < 0.01\); c, \(P < 0.001\)).

Figure 3. Effect of estradiol-17β on protein content of fat body cytosol of male and female pupae of A. mylitta. Three consecutive injections were given to 130, 135 and 140-day-old pupae. Vertical bars represent the standard error of the mean (a, \(P < 0.05\); b, \(P < 0.01\); c, \(P < 0.001\)).

Among the several lipogenic enzymes, NADP⁺-linked malate dehydrogenase is well known to be estrogen-sensitive in invertebrates. In this study we have demonstrated that MDH activity in both the subcellular fractions of fat body cells in A. mylitta is inducible by exogenous application of 17-β estradiol. This response is expressed by about a maximum of 65.66% to 135.38% (in male and female pupae respectively) increase in the specific enzyme activity mainly for mMDH. The increase can be attributed mainly to de novo synthesis of the enzyme.

Hormonal induced and/or enhanced enzyme activity appears to be a result of an increase in enzyme mass in silkworm and other organisms. On the other
Figure 4. Effect of estradiol-17β on protein content of fat body mitochondrial fractions of male and female pupae of A. mylitta. Three consecutive injections were given to 130, 135 and 140-day-old pupae. Vertical bars represent the standard error of the mean (a, P < 0.05; b, P < 0.01; c, P < 0.001).

hand, vertebrate estrogen at higher doses (10 and 50 μg) significantly decreased the NADP⁺-linked cMDH activity by about 33.77% (on an average, irrespective of sex and doses of estrogen) in fat body cells. It is important to note that E₂-induced decrease in NADP⁺-dependent cMDH in fat body cells of A. mylitta on 150th day, during pupal diapause is indicative of lipolysis and could be occurring due to concomitant enhancement in the level of plasma octopamine as reported previously. Changes in hemolymph octopamine levels have been reported under a number of specific circumstances performing several biochemical changes controlling short-term lipid and carbohydrate metabolism on being released as a part of an ‘arousal mechanism’ to stressful circumstances. However, during lipolysis the energy requirement of cells is increased. Hence, this E₂-induced increase in energy requirement of the cells is most likely by a concomitant rise in fat body mMDH activity (an enzyme of TCA cycle) which may enhance ATP⁺ production.

In the present study, significant rise in protein levels of cytosolic as well as mitochondrial fraction with all the doses of E₂ used was recorded in both the sexes of A. mylitta together with higher level of protein content in hemolymph plasma after E₂ treatment which are indicative of increased synthesis or decreased degradation of protein in the fat body tissue as in B. mori. There are evidences that exogenous steroids (estrogen/ecdysone) stimulate protein synthesis and increase oxygen consumption in insect tissues including the mulberry silkworm B. mori. In control lots, protein content in both the mitochondrial and cytosolic fractions of fat body remained significantly (P < 0.001) higher (73.53% and 222.61%) in female diapausing pupa of A. mylitta than that of males (Figures 3 and 4) as found in non-diapausing larvae of A. mylitta. This increased protein level in female pupae may be due to synthesis of femalespecific proteins in fat body cells. All the doses of estradiol 17-β significantly (P < 0.05-0.001) elevated the protein level in both the sexes as well as in both the sub-cellular fractions of fat body tissue compared to non-treated controls. Also, all the doses of E₂ showed the same magnitude of enhancement in the protein content of mitochondrial and cytosolic fractions of fat body cells.

Thus, estrogen-induced effect on NAD⁺ and NADP⁺-linked enzyme systems and protein content of fat body cells once again strongly supports the steroid hormone effect on early moth eclosion in A. mylitta by triggering tissue metabolism in advance for pupal-adult transformation, thereby controlling the diapause regulatory mechanism. In addition, a dose-dependent enhancement in malic enzyme activity by E₂ treatment indicates possible receptor-mediated hormonal action associated with receptor saturation and physiologic response in the fat body cells of this insect. Further studies on the search for estrogen receptors in different organs of A. mylitta will confirm such assumption.

Modelling of light modulation processes in D85N bacteriorhodopsin

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A simplified model for the complex photocycle of the D85N genetic variant of the bacteriorhodopsin (bR) protein molecule is presented. Steady state population densities of the various intermediate states of the molecule induced by photo-absorption of modulation light beam are obtained using the rate equations approach. All-optical modulation of various probe signals at wavelengths corresponding to absorption peaks of each of the intermediate states by a pump signal at 570 nm is presented in the form of optical densities. The analysis presented here is useful for designing the molecular spatial light modulators using D85N variant of bR molecules.

Recent years have witnessed dramatic progress in investigating novel materials for all-optical signal processing and data storage. The photochromic protein bacteriorhodopsin (bR) which is found in the purple membrane of Halobacterium halobium, has emerged as an excellent material for bio-molecular photonic applications due to its unique advantages. bR absorbs light in a bacterium and undergoes a complex photocycle that generates intermediate states with absorption maxima spanning the entire visible region of the spectrum. It has a high quantum efficiency of generating light into a state change and large absorption cross-section. The crystal-like architecture leads to high stability and makes it

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