VITELLOGENESIS IN A LADY-BEETLE, Coccinella septempunctata in Relation to the Aestivation-Diapause

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Abstract—Yolk-protein precursor, vitellogenin, synthesis and effects of juvenile hormone analogue on vitellogenesis were studied in a lady-beetle, Coccinella septempunctata, which enters aestivation-diapause in the adult stage. In the pre-diapause period, total protein synthesis in the fat body was relatively high but vitellogenin synthesis was negligible. During diapause, total protein synthesis declined to a low level (but detectable) and vitellogenin synthesis was not detected at all. In the course of diapause termination, total protein synthesis recovered gradually, but vitellogenin synthesis did not occur before feeding. Juvenile hormone analogue application to a diapausing female induces vitellogenin synthesis and consequent oviposition. Sensitivities to juvenile hormone analogue in the stimulation of vitellogenin synthesis depended on the stage of diapause: pre-diapause period, diapause period and post-diapause period. The time lags between juvenile hormone analogue application and vitellogenin induction in the haemolymph were shorter in the pre- and post-diapause period than that in diapausing females.

Key Word Index: Diapause, aestivation, lady beetle, Coccinella septempunctata, vitellogenesis, vitellogenin, juvenile hormone, methoprene

INTRODUCTION

Some coleopteran and hemipteran insects enter diapause in the adult stage, usually under short-day conditions. In these insects, ovarian development is arrested in diapausing females, and the diapause is, therefore, called a reproductive diapause. This adult diapause has often been demonstrated to be due to a reduction of juvenile hormone secreted from the corpus allatum. In many diapausing Coleoptera, application of juvenile hormone or juvenile hormone analogue can induce both vitellogenesis and oviposition (Bowers and Blickenstaff 1966; Connin et al., 1967; Hodek et al., 1973; Mohamed Ali, 1979; Ashida, 1980). In Henosepilachna beetles (Kono, 1980) and in the Colorado potato beetle, Leptinotarsa decemlineata (de Loof and de Wilde, 1970), however, application of juvenile hormone (analogue) elicited vitellogenesis but failed to cause complete oocyte maturation. In these two insects, inactivation of both neurosecretory cells and corpus allatum might induce diapause, and a neurosecretory factor originating in the brain might stimulate vitellogenesis along with juvenile hormone (de Loof and de Wilde, 1970).

The seven-spotted lady beetle Coccinella septempunctata biueki Mulsant enters into diapause in the adult stage in the summer in central Japan (Sakurai et al., 1981a,b; 1982). It has been demonstrated that diapause in C. septempunctata is induced in long-day conditions, i.e. the photoperiodic response of this insect is of the short-day type (Okuda and Hodek, 1983). This diapause in C. septempunctata was suggested to be induced by a lack of corpus allatum activity judging from the changes in corpus allatum size (Sakurai et al., 1981b). Juvenile hormone (analogue) application can induce vitellogenesis and oviposition, and therefore this insect is not grouped in the type of the Colorado potato beetle but in that of many insects in which diapause depends mainly on juvenile hormone. Recently, Zhai et al. (1984) studied on vitellogenin-synthesis induction by a juvenile hormone analogue in C. septempunctata. They showed that vitellogenin synthesis was detected mainly in the fat body and also in the ovary in adult female, and that vitellogenin synthesis was induced by juvenile hormone analogue application to non-reproductive females. Sakurai et al. (1987) observed an induction of a haemolymph protein specific to reproductive females and the disappearance of a diapause-specific haemolymph protein after juvenile hormone analogue application.

In this paper, we focused on C. septempunctata female reproduction particularly on the synthesis of the yolk-protein precursor, vitellogenin, in the fat body, in relation to diapause. The reaction of diapausing female beetles to application of juvenile hormone analogue was also investigated.

MATERIALS AND METHODS

Animals

Pupae of a lady beetle, Coccinella septempunctata were collected in the field at Mie-ken in central Japan from late April until June, and transferred to a long-day (18 h light-6 h dark) laboratory condition at 25 ± 2°C. After the emergence of females, aphids (mainly Acyrthosiphon pisum) were given as food. About 90% of the pupae collected in the field in mid-May entered diapause. During the diapause,
beetles were kept at 25°C in about 60% r.h. and long-day conditions.

Chemicals

[^14C]Amino acid mixture (57 mCi/milliatom carbon) was purchased from the Radiochemical Center (Amersham, U.K.). Juvenile hormone analogue, methoprene (ZR-515, racemic mixture) was a gift of Dr Shimada (Otsuka Chemical Co.). Reagents for polyacrylamide gel electrophoresis came from Bio-Rad (U.S.A.). Other chemicals used were analytical grade.

Collection of haemolymph and dissection of lady-beetles

Haemolymph was collected from the legs of females by reflex bleeding. Protein composition of haemolymph collected by flushing was demonstrated by sodium dodecyl sulphate—PAGE to be completely identical with that of haemolymph bled from the neck membrane (data not shown). Fat body was dissected from female abdomens in the cold under phosphate-buffered saline (0.02 M phosphate buffer pH 7.2—0.15 M NaCl).

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulphate was performed as described by Laemmli (1970) using an acrylamide gradient (2.5 to 15%) gel in 0.375 M Tris—Cl buffer (pH 8.8), 0.1% sodium dodecyl sulphate with 3% stacking gel in 0.125 M Tris—Cl buffer (pH 6.3) containing 0.1% sodium dodecyl sulphate. Samples were dissolved in about same volume of 0.1 M Tris—Cl buffer (pH 8.8) containing 2% sodium dodecyl sulphate, 5% 2-mercaptoethanol and 10% glycerol, and heated in boiling water for 2—3 min. Electrophoresis was carried out in the 0.01 M Tris—Cl, pH 8.5, 0.25 M NaCl, 1 mM phenylmethylsulphonyl-fluoride, 0.1% Triton X-100, 0.1% sodium deoxycholate and 0.1% sodium dodecyl sulphate). Homogenates were centrifuged at 5,000 g for 5 min. The aqueous layer was removed and stored at —70°C as crude tissue extract.

For counting the radioactivity incorporated into total protein, 10 µl of crude tissue extract was put on a 74 mm disc of Whatman 3 MM filter paper. After drying slightly, discs were put in cold 10% trichloroacetic acid (TCA) and washed 3 times with cold 5% TCA, once with ethanol—ether (3:1, v/v) and once with ether. Discs were dried in air, put into vials with 5 ml of a toluene-base scintillator and counted with a LKB scintillation counter (type 1219).

For quantitative precipitation of vitellogenin from the crude tissue extract, rocket immunoelectrophoresis IE was performed. Ten µl of extract and 1 µl of carrier Vitellin (5 µg) were mixed and applied onto a 1% agarose plate containing 2% anti-vitellin serum. Radioactive vitellogenin in the extract reacted with anti-vitellin serum in the agar during electrophoresis and precipitated quantitatively as a rocket. After electrophoresis, the gel was washed 5 times with phosphate-buffered saline, stained for few minutes with Coomassie blue and destained. Stained rockets were cut with a blade and melted with 2 ml water at 100°C in scintillation vials. Radioactivity was counted after mixing with 10 ml aquasol scintillator.

RESULTS

Changes in haemolymph protein and vitellogenin during adult diapause

Haemolymph collected from adult females in pre-diapause, diapause and post-diapause was analysed by sodium dodecyl sulphate-PAGE (Fig. 1). Protein patterns separated thus did not change through the stages of development except bands P1 to P4, which were demonstrated to be subunits of both vitellogenin and vitellin and to be identical with vitellogenin and vitellin in immunological reaction (Zhai et al., 1984). Vitellogenin titres were determined by scanning the stained gels (Fig. 2). Vitellogenin was detected in the haemolymph of adult females in pre-diapause stage (day-5 after emergence), at a very low level (about 5 µg/µl at maximum). This level was one-sixth of that in the haemolymph of reproductive (egg-laying) fe-
Fig. 1. Sodium dodecyl sulphate PAGE of haemolymph proteins and crude egg extract. Haemolymph was collected from females in pre-diapause (0, 5 and 10 days), diapause (30, 45 and 90 days) and after diapause (PD, 100 days after adult emergence), and analysed on gels (2.5 to 15% gradient) together with the crude egg extract (CEE). P1 to P4 indicate the subunit bands of vitellin and vitellogenin.
Fig. 4. Sodium dodecyl sulphate-PAGE of diapausing female haemolymph after methoprene treatment. 10 μg of methoprene was applied topically to females 30 days after emergence (diapausing). Haemolymph was collected each day after methoprene treatment as shown at the top of each lane of the gel, and applied on sodium dodecyl sulphate-PAGE analysis. Crude egg extract (CEE) was also run in the same gel.
Vitellogenesis of lady-beetle males. At the cessation of feeding (i.e. diapause initiation), from the 5th day to 10th day after emergence vitellogenin disappeared from the haemolymph. During diapause (day-30, 45 and 90 after emergence), vitellogenin was not detected at all.

Some females kept at 25°C start to feed again on aphids about 90-100 days after emergence. In the haemolymph of females in this post-diapause stage, vitellogenin became detectable if the beetles were fed. The vitellogenin titre in the haemolymph of post-diapause females rose to 30 µg/µl, and remained rather constant at this level during the reproductive (ovipositing) period after diapause (data not shown).

Although vitellogenin was detectable in pre-diapause stage, ovarian development, i.e. accumulation of vitellin in the oocytes, was not observed in this stage. During diapause, no visible changes were recognized in the ovary. After diapause termination, when females start to feed on aphids, accumulation of the yolk vitellin in the ovaries was synchronized with the increase of vitellogenin titre in the haemolymph.

Vitellogenin and total protein synthesis in fat body during pre-diapause, diapause and after diapause

Fat bodies dissected from females in pre-diapause, diapause and after diapause were incubated in vitro with 14C-amino acid mixture and radioactivities incorporated into total protein (TCA-insoluble fraction) and vitellogenin (specific precipitate against anti-vitellin serum) were measured (Fig. 3). The change of vitellogenin synthetic activity in the fat body was well correlated with that of vitellogenin titre in the haemolymph (Figs 2 and 3). Vitellogenin synthesis was at a very low but significant level in the fat body of pre-diapause females (at day 5 after emergence). During diapause, vitellogenin synthetic activity in the fat body was undetectable. After the termination of diapause, vitellogenin synthetic activities increased quickly. Total protein synthesis, on the other hand, was detected throughout the stages of diapause (Fig. 3). The activity of total protein synthesis was high before and after diapause, but low during diapause. Particularly in 5-day old prediapause females, total protein was synthesized actively, while the activity of vitellogenin synthesis was very low. At 10 days after emergence, when females ceased feeding and the fat body became very hypertrophied, protein synthesis in fat body commenced to decrease. In diapause at day 30, when the fat body was at its biggest size, total protein synthesis recorded the minimal activity. During further development of diapause, protein synthesis recovered gradually and finally reached a maximal level in the post-diapause development. The ratio of vitellogenin synthesis to total protein synthesis was about 15-20% in reproductive females after diapause.

Induction of vitellogenin in the haemolymph and diapause termination by juvenile hormone analogue

Various doses of juvenile hormone analogue, methoprene, were applied topically to diapausing female beetles (45 days after emergence under long-day conditions). Diapause termination was induced by the higher doses of methoprene (over 1 µg/insect). Those females started and continued to feed and oviposit, which meant the complete termination of diapause. Lower doses (less than 0.1 µg) could not induce complete diapause termination, but could
induce a vitellogenin titre in the haemolymph. In lower doses it took a longer time for vitellogenin to reach its normal titre (25 ± 5 μg) in the reproductive female haemolymph. Vitellogenin induction in the haemolymph depended on the methoprene doses in the wide range from 0.01 to 10 μg (data not shown).

Changes of vitellogenin titre in the haemolymph after juvenile hormone analogue treatment

Methoprene (10 μg) was applied topically to diapausing females at day 30 after emergence. Haemolymph was collected from the treated female every day at 1–4 days and at given intervals 6–14 days after treatment. Haemolymph was applied to sodium dodecyl sulphate-PAGE (Fig. 4), and the protein concentration was analysed by densitometric scanning as described under Materials and Methods (Fig. 5). The subunits of vitellogenin (P1 to P4) were detected already at 1 day and observed clearly 2 days after treatment. Vitellogenin titre increased steeply at days 2 to 3 after treatment and after that did not show any big change. A few haemolymph proteins other than vitellogenin changed significantly, but most proteins were not affected by methoprene treatment.

Induction of vitellogenin and total protein synthesis by juvenile hormone analogue treatment

Fat bodies dissected from females treated with methoprene (10 μg) were incubated in vitro with 14C-amino acids, and vitellogenin and total protein synthetic activities were counted. Figure 6 shows vitellogenin synthetic activity in the fat body after methoprene treatment. The radioactivity in the precipitate of fat body extract with anti-vitellin serum decreased already at 1 day and reached a maximum at 4 days and then decreased at 6 days after methoprene treatment. This change in vitellogenin synthetic activity was well correlated with the vitellogenin induction in the haemolymph after methoprene treatment. This suggests that vitellogenin synthesized in the fat body is secreted into the haemolymph without time lag.

Total protein synthesis in the fat body after methoprene treatment is shown in Fig. 6. The time course was slightly different from that of vitellogenin synthesis. Total protein synthesis reached a maximum 1–2 days earlier than vitellogenin synthesis after methoprene treatment.

Change in sensitivity to methoprene during diapause

Methoprene (1 μg) was applied topically to females in various stages of diapause. Haemolymph was collected every day after treatment, and vitellogenin titre was determined by densitometric scanning of stained sodium dodecyl sulphate-PAGE gel (Table 1). Vitellogenin induction in the haemolymph by methoprene changes from stage to stage. In prediapause females (day 0, 3 and 14 after emergence), vitellogenin was detected first at 1 day (24 hr) after treatment and increased quickly particularly in the females at the age of 3 and 14 days. In the diapausing females (day 30 and 60), vitellogenin appearance was delayed one more day than in the pre-diapause females. Vitellogenin titre, however, caught up to the level of prediapause females by day 3 after methoprene treatment. On the other hand, at 90 days after emergence, vitellogenin was induced 1 day after methoprene treatment to the level of post-diapause (reproductive) females, although those females were still inactive and in a pre-feeding stage.

DISCUSSION

Changes in vitellogenin titre in the haemolymph and vitellogenin synthesis in the fat body during adult diapause

Vitellogenin was detected significantly in the haemolymph at day 5 after emergence (but not at day 0, 3 and 10), although vitellogenin synthesis was very low in the pre-diapause stage (day 0, 5 and 10). This fact is in accordance with field observations in central Japan which showed 10% of C. septempunctata females had developed (yolk deposited) ovaries before entering aestivation diapause (Okuda, unpublished data). Diapausing females caught in the field did not have developed ovaries. This suggests that vitellin accumulated in the ovary in the pre-diapause stage was resorbed during the onset of diapause. Total protein synthesis was activated to a rather high level in prediapause. This activation of protein synthesis or partial development of vitellogenesis in the period between emergence and commencement of diapause might be controlled by juvenile hormone, because the volume of corpus allatum was observed to increase to an intermediate size in this period (Sakurai et al., 1981b). A similar phenomenon including a small increase of juvenile hormone has been reported in pre-diapause females of Leptinotarsa (DeKort et al., 1981) and Oncopelthus (Rankin and Riddiford, 1978).

During diapause, neither vitellogenin titre in the haemolymph nor vitellogenin synthesis in the fat body was observed at all. Total protein synthesis in

Table 1. Vitellogenin induction by methoprene during the prediapause and diapause period of lady beetle, Coccinella septempunctata, adult females

<table>
<thead>
<tr>
<th>Adult age (days after emergence)</th>
<th>d0*</th>
<th>Vitellogenin titre (μg/d) after methoprene treatment</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>d1</td>
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<tr>
<td>0</td>
<td>0</td>
<td>1.2 ± 1.0*</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>2.4 ± 2.5</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>2.0 ± 2.3</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>18.5 ± 2.5</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>24.7 ± 9.8</td>
</tr>
<tr>
<td>90</td>
<td>0</td>
<td>17.2 ± 12.5</td>
</tr>
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</table>

*Just before methoprene treatment.

†Mean ± standard deviation (n = 10).

Beetles were treated topically with 1 μg methoprene dissolved in acetone. Haemolymph samples from individual beetles were applied on sodium dodecyl sulphate-PAGE. Vitellogenin concentration was determined by scanning of stained gels and calibrated with standard vitellin.
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Fig. 5. Change of vitellogenin titre in the haemolymph of diapaus- 
ing females after methoprene treatment. Haemolymph was collected from females which had been treated with methoprene (10 µg) topically 30 days into diapause. Vitellogenin titre was determined as in Fig. 2.

the fat body also declined to a low level in diapause females. In diapausing beetles flight muscles have been reported to degenerate (Sakurai et al., 1982). A drastic drop in respiration in diapause beetles (Sakurai, 1969; Sakurai et al., 1981a) might be related to those changes in the metabolic activities of fat bodies and muscles.

In the late diaposing stage about 90 days after emergence, females are very close to diapause termination, but are not feeding yet. The activity of protein synthesis in the fat body recovered to almost the same level as that of reproductive females, although vitel-

logenin synthesis was not observed. After diapause has terminated, beetles start to feed on aphids, and the vitellogenin titre in the haemolymph increases.

The effect of juvenile hormone analogue application on vitellogenin induction in aestivating females

Topical application of methoprene to aestivating females C. septempunctata can stimulate vitellogenin synthesis in the fat body and secretion into the haemolymph, and finally terminate diapause completely, so that the treated females started to feed and oviposit. Sakurai et al. (1987) reported the induction of a specific protein band corresponding to vitellogenin by an application of methoprene to diapausing C. septempunctata. They also showed that diapause-specific proteins disappeared by methoprene application. In our analysis of haemolymph proteins by sodium dodecyl sulphate PAGE, we could not find any such diapause-specific proteins in this species. Vitellogenin synthesis induction by a juvenile hormone analogue in C. septempunctata has been showed clearly in in vivo and in vitro system by Zhai et al. (1984). Similarly in many coleopteran insects juvenile hormone (analogue) application to diapausing females was reported to induce vitellogenesis and oviposition (Bowers and Blickenstaff, 1966; Connin et al., 1967; Hodek et al., 1973; Mohamed Ali, 1979; Ashida, 1980). In the Colorado potato beetle, Lepinotarsa, on the other hand, juvenile hormone application induced vitellogenesis and oviposition in allatectomized long-day females, but failed to do so in non-operated and allatectomized short-day females (de Loof and de Wilde, 1970; Sehnal and Skuhravy, 1976). In 28-spotted lady-beettle, Henosepilachana vigintioctopunctata, methoprene treatment could induce vitellogenin in the haemolymph and ovarian development, but the induction of oviposition and complete termination of diapause was not observed (Kono, 1986; Kono and Ozeki, 1987). In C. septempunctata, a single dose of methoprene (even 1.0 µg) could lead to vitellogenin induction, ovary development and complete termination of diapause. Those observations suggest that there are two groups in these coleopteran insects based on the differences in response to exogenous juvenile hormone (analogue); the Colorado potato beetle-type, and the general type. In the former, juvenile hormone alone cannot terminate diapause and termination needs both a neurosecretory factor and juvenile hormone, while in the latter type, juvenile hormone alone terminates diapause. In the seven-spotted lady-beetles. Juvenile hormone (analogue) can terminate diapause completely, but it has not been elucidated whether (1) any neurosecretory factor is necessary to terminate diapause, (2) a neurosecretory factor is always secreted even during the diapause, or (3) juvenile hormone can activate the neurosecretory cells to secrete the factor. This problem might be solved by an in vitro experiment to show activation of vitellogenin synthesis in the fat body in medium supplemented with juvenile hormone or some factors such as a brain extract.

Although a single dose of methoprene induces complete vitellogenesis in C. septempunctata, the sensitivity to methoprene changes in the processing of diapause, i.e. pre-diapause, diapause and late di-

Fig. 6. Changes of total protein and vitellogenin synthesis in the fat body after methoprene treatment. Fat bodies were dissected from females in various stages after methoprene (10 µg) treatment at 30 days into diapause, and incubated in vitro. Detail procedure for measuring total protein and vitellogenin synthetic activities was described in Materials and Methods. Each point indicates the activity of one incubation of three fat bodies.
apause (Table 1). Adult females in diapause showed the least sensitivity, i.e. the longest time lag between methoprene application and vitellogenin synthesis. This alteration of sensitivity to methoprene in fat body vitellogenin synthesis seemed to be related to the activity of protein synthesis in the fat body (Fig. 3), so that when the activity of protein synthesis was relatively high, females showed high sensitivity to methoprene. This suggests that for active synthesis of vitellogenin, the fat body should have developed organelles such as ribosomes and endoplasmic reticulum for protein synthesis. Time lags between juvenile hormone analogue application and vitellogenin titre might be needed for building up the apparatus for protein synthesis from the hypertrophic-inactive fat body to active protein-synthetic fat body.

A similar phenomenon was reported after vitellogenesis of Locusta migratoria, in which females allatectomized chemically with precocene initiated vitellogenin synthesis in the fat body at day 2 and at day 1 after the primary and secondary treatment of methoprene, respectively (Chinzei and Wyatt, 1985). They suggested these differences in fat body sensitivity to methoprene might depend on the difference in the development of organelles for protein synthesis between the primary and secondary treatment with the hormone (Chinzei et al., 1982). We suggest that methoprene applied to diapausing female beetles may first induce fat body reconstruction to activate protein synthesis and then initiate specific vitellogenin synthesis. In fact, our observations on protein synthesis in the fat body cultured in vitro indicate that stimulated total protein synthesis preceded vitellogenin synthesis (Fig. 6).

In the diaposing insect, the fat body is hypertrophied. The fat body's metabolic activity including protein synthesis are at a minimum in the hypertrophied fat body. The fat body in diaposing insects may be a storage tissue for nutrient reserves. There seems to be a reverse and competitive relationship of function between storage and biosynthesis in fat body. Conversion of fat body function from a metabolically active tissue to a storage tissue and the alteration of sensitivity to methoprene might depend on the difference in the development of organelles for protein synthesis.

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REFERENCES


Acta ent. Bohemoslov. 80, 74-75.


Sehnal F. and Sluhrav V. (1976) Effects of juvenoids on Lepinotarsa decemlineata (Col., Chrysomblidae) and
