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Dinocampus (= *Perilitus*) *coccinellae* teratocyte-specific polypeptide: its accumulative property, localization and characterization

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Abstract

Dinocampus (= *Perilitus*) *coccinellae* (Braconidae: Hymenoptera) teratocytes synthesize a teratocyte-specific polypeptide (TSP) with a high molecular weight of 540 kDa. The TSP has a tendency to accumulate in the teratocyte cells without release after synthesis (Okuda and Kadono-Okuda, 1995), which was confirmed in this study. Pulse-chase fluorography indicated that teratocytes at a younger stage (6 days after parasitization)secreted negligible TSP into the medium after synthesis, while teratocytes at an older stage (11 days after parasitization)secreted the synthesized products into the medium, although the amount released was still low. Western blot with anti-TSP serum showed that only a small amount of TSP appeared in the parasitized host hemolymph, even when TSP synthesis by teratocytes was actively taking place, which also supported the accumulative nature of TSP. The immunoelectronmicroscopic studies revealed that the TSP was localized specifically in high electron-dense vacuoles. Lectin blot analysis identified TSP as a high mannose glycoprotein. The amino acid composition of the major subunit of the TSP was quite similar to that of nutritive proteins such as vitellogenin and storage proteins of some insects. These characterization data, together with the accumulation property of the TSP indicates that *Dinocampus* teratocyte primarily plays a nutritive role for the developing parasitoid larvae. TSP exhibited esterase activity, which indicates that TSP may have an additional function in the host-parasitoid reaction.

Keywords: Dinocampus coccineallae; *Coccinella septempunctata*; Teratocytes; Host-parasitoid relationship; Braconidae; Teratocyte-specific polypeptide; Esterase

1. Introduction

The role of embryonic serosa is not well understood. A possible function is the secretion of cuticular layers which together with a chorion, make the egg structure stronger (Wigglesworth, 1972). This may be supported by the fact that fertilized *Heliothis virescens* eggs are more resistant to desiccation than infertile eggs lacking serosa (Strand et al., 1986). Another secondary role may be as a nutritive source. For example, in the silk moth, *Bombyx mori*, serosal cells and the yolk cells are consumed by the embryo before hatching (Kanda, personal communication). In some hymenopteran wasps, such serosal cells are not ingested during embryogenesis and can be found in the host hemocoel after the egg hatching of the parasitoid larva. These post embryonic serosal cells are often called “teratocytes” and may play several roles in the host-parasitoid reaction, although the biological function may differ one system to another (Dahlman, 1990). In some systems, teratocytes remain small but they disrupt host immunity (Kitano et al., 1990). In others, they exert a suppressive effect on host hemolymph protein production by fat body (Zhang et al., 1997) or they may have a regulatory effect on host metamorphosis (Zhang and Dahlman, 1989; Wani et al., 1990; Strand and Wong, 1991; Pennacchio et al., 1994).

During parasitism of *Coccinella septempunctata* by *Dinocampus coccinellae*, it is suggested that teratocytes

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may be primarily nutritive, since they are consumed by the parasitoid larvae (Okuda and Kadono-Okuda, 1995). Interestingly, these teratocytes become hypertrophic (Sluss, 1968; Kadono-Okuda et al., 1995) with a maximum diameter of 820 μm. Exclusive synthesis of teratocyte-specific polypeptide (TSP) with a high molecular weight of 540 kDa further supports the above assumption (Okuda and Kadono-Okuda, 1995). In this paper we confirm the accumulative property of TSP and investigate localization and characterization of this protein.

2. Materials and methods

2.1. Insects

Dinocampus coccinellae and *Coccinella septempunctata* were reared in the laboratory using the method of Okuda and Kadono-Okuda (1995). Adult wasps were fed a 30% sucrose solution until used for parasitization. Host coccinellids, reared under diapause averting conditions at short day (12L:12D) and 25°C (Okuda and Hodek, 1983), were parasitized 7 days after adult eclosion by *D. coccinellae*. Teratocytes were sampled either 6 or 11 days after parasitization using the methods previously described by Okuda and Kadono-Okuda (1995).

2.2. Polyacrylamide gel electrophoresis (PAGE), western blot and lectin blot

SDS-PAGE (2.5–20% gradient gel or 7.5% gel) was performed as described by Laemmli (1970). For Western blot analysis, host hemolymph and teratocyte crude extracts were subjected to SDS-PAGE (7.5% gel) followed by staining with Coomassie Brilliant Blue (CBB) or immuno staining (Okuda and Kadono-Okuda, 1995). Host hemolymph was collected from the legs by reflex breeding (Okuda and Chinzei, 1988). Antisera to partially purified TSP were raised in a rabbit. For lectin blot analysis, teratocyte crude extracts were subjected to SDS-PAGE (7.5% gel) followed by staining with a peroxydase conjugated lectins kit (Honen Company).

2.3. Pulse-chase fluorography

Teratocytes sampled from parasitized host at days 6 and 11 after parasitization were incubated in Grace's medium (without methionine) containing 1 μCi of ^{35}S methionine (47.1 TBq/mmol, Amersham) for 15 min at 25°C. The pulse-labeled teratocytes were then transferred into fresh Grace's medium (with non-radiolabeled methionine) for chase at 25°C. In each chase-incubation, approximately 500 immature teratocytes collected on day 6 and 30 matured teratocytes collected on day 11 were used, respectively. No destruction of the matured teratocytes during the pulse-chase incubation was confirmed by binocular observations. The samples from homogenized teratocytes and incubated media were subjected to SDS-PAGE (2.5–20% gradient gel) followed by fluorography as described by Okuda and Kadono-Okuda (1995).

2.4. Preparation of teratocyte samples for light microscopy

After being anesthetized with CO_2, the whole body of the parasitized host was fixed by immersion in 10% formaldehyde. Fixed samples were rinsed in 70% ethanol, progressively dehydrated in graded ethanols and in xylene and embedded in paraffin. Paraffin sections of 6–7 μm thickness were cut and stained with Delafield's hematoxlin and eosin.

2.5. Immunoelectron microscopy

Teratocytes sampled from parasitized hosts at day 8 after parasitization were fixed with a mixture of 2% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M cacodylate buffer pH 7.2 for 1.5 h at laboratory temperature. These cells were washed in 0.1 M cacodylate buffer pH 7.2 for 0.5 h, were then dehydrated in 50 and 75% ethanol (10 min each) and embedded in LR White for 20 h at 60°C. Ultrathin sections were collected on nickel grids and treated with glycine in 0.05 M sodium phosphate buffer (pH 7.4) for 10 min. The primary antibody used was an antiserum to TSP (1:100, 1:300 and 1:1500). Sections were treated for 1 h at laboratory temperature, then washed in 0.05 M sodium phosphate buffer and treated with gold-labeled (10 nm)secondary antibody (1:40) for 1 h, washed, double-stained with aqueous uranyl acetate and lead citrate, and examined with JOEL electron microscope.

2.6. Esterase zymography

Host tissues or teratocytes were homogenized in 50 μl of a sucrose solution (0.5 M sucrose, 0.05 M potassium phosphate buffer with pH 6.8), centrifuged at 3000 ***g*** for 15 min, and the supernatants stored at − 70°C until use. Acrylamide gel columns in glass tubes of 5 mm in diameter and 70 mm in length, were prepared for vertical disc-gel electrophoresis. Electrophoresis was performed at 5°C. For the esterase zymography, α-naphtyl acetate was used as the substrate, with Fast Blue RR-salt in a 5 mM potassium phosphate buffer used as a staining reagent, and 50% ethanol as a destaining solution (Sasaji and Ohnishi, 1973).

2.7. Microplate enzyme assays

The buffer used for enzyme assays was 0.05 M sodium phosphate, pH 7.4, containing 10% sucrose.

Esterase activity in a crude teratocyte extract was determined on a microplate with two substrates, p-nitrophenyl acetate (p-NPA) for general esterases as reported by Grant et al. (1989) and methyl n-heptylthioaceto-thioate (HEPTAT) for juvenile hormone esterases as reported by McCutchen et al. (1993). Briefly, 293 μl of the buffer was preincubated at 25°C for 5 min, 0.025% 5,5′-dithiobis(2-nitrobenzoic acid) was supplemented to the buffer for junvenile hormone esterases. Then, 2 μl of substrate solution (75 mM p-NPA or 30 mM HEPTAT in ethanol) and 2.5 and 5 μl of crude teratocyte extracts (2.43 mg/ml in protein content tested by Bradford assay) were added to the buffer, and changes of absorbance at 405 nm were recorded for 2 min on a microplate reader (SPECTRA-Max by Molecular Devices, Menlo Park, CA, USA.). The hydrolyzing rate was calculated using an analysis software SOFTmax PRO (Molecular Devices).

3. Results

3.1. Teratocyte-specific polypeptide (TSP) in the host hemolymph

Hemolymph samples from parasitized (day-11 after parasitization) and non-parasitized hosts were subjected to SDS-PAGE and these protein patterns were visualized by Coomassie Brilliant Blue (CBB) and immuno staining (IMS) respectively (Fig. 1-left columns). The electrophoreograms showed that there were no qualitative differences in protein profile between the parasitized and non-parasitized host hemolymph samples, except for the vitellogenin bands. Vitellogenin synthesis by the host fat body was inhibited by parasitism. Western blotting with anti-TSP serum, however, clearly visualized the 94 kDa TSP subunit existing only in the parasitized host hemolymph. These results indicated that only a small amount of TSP appeared in the host hemolymph, even during the period when TSP was actively synthesized (Fig. 4; Okuda and Kadono-Okuda, 1995).

Similarly, when crude teratocyte extracts were sampled from hosts 6 and 11 days after parasitization and subjected to SDS-PAGE followed by CBB and immuno stainings (Fig. 1-right columns), the results indicated that the TSP content in teratocytes increased with age of the parasitoid.

3.2. Pulse-chase fluorography

The above results, together with a previous report (Okuda and Kadono-Okuda, 1995), suggested that the synthesized TSP accumulates in the teratocyte cells with a slight release into the host hemolymph. To confirm this, pulse-chase fluorography was carried out with teratocytes sampled at different stages, day 6 (Fig. 2) and day 11 (Fig. 3) after parasitization. The fluorogram showed that the small teratocytes day 6 after parasitization secreted a negligible amount of TSP, while the more advanced teratocytes collected on day 11 after parasitization apparently secreted the de novo synthesized products into the medium, though the amount that appeared in the medium was still small. For comparison, pulse-chase fluorography was carried out similarly for fat body taken from reproductive non-parasitized host females (Fig. 4). Vitellogenin, a nutritive protein, was synthesized by the fat body and the two subunits of this

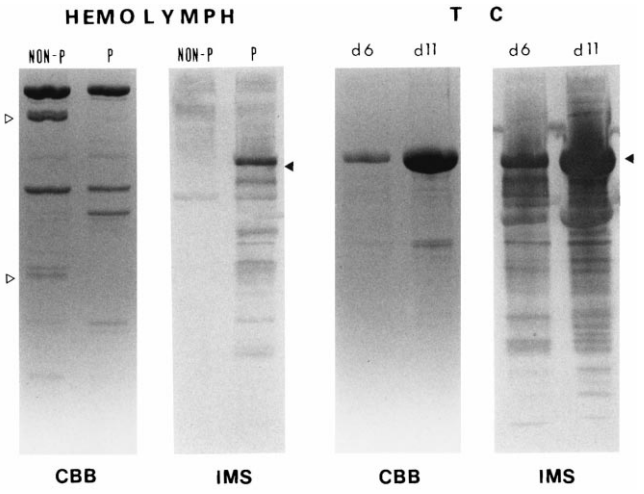

Fig. 1. Western blot of extracts from *C. septempunctata* host hemolymph and teratocyte of *D. coccinellae*. Teratocyte extracts (TC) and hemolymph (0.5 μl) from parasitized (P) (day 11 after parasitization) and non- parasitized (NON-P) hosts were respectively subjected to SDS-PAGE and followed by Coomassie Brilliant Blue (CBB) and immuno (IMS) staining. d6; teratocytes sampled at day 6 after parasitization, d11; teratocytes sampled at day 11 after parasitization. Open arrows indicate two vitellogenin subunits and a closed arrow indicates 94 kDa TSP subunit.

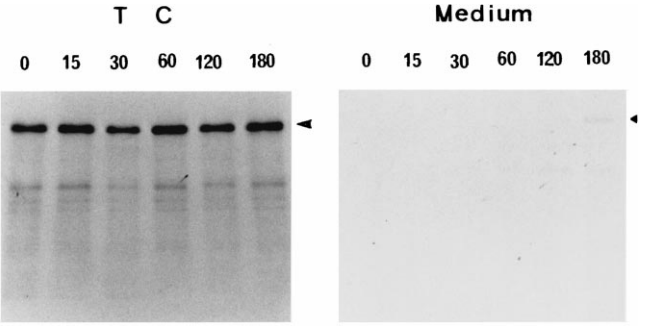

Fig. 2. Pulse-chase fluorography of teratocytes of *D. coccinellae* sampled from hosts (*C. septempunctata*) at early parasitism. Teratocytes sampled from day 6 after parasitization were incubated in Grace's medium (without methionine) with ^{35}S methionine for 15 min and these teratocytes were transferred into fresh Grace's medium (with methionine) followed by sampling at each time after chase-incubation. The number above each gel indicates the time (min) after chase-incubation. Each extracted sample was subjected to SDS- PAGE and followed by fluorography. An arrow indicates 94 kDa TSP subunit.

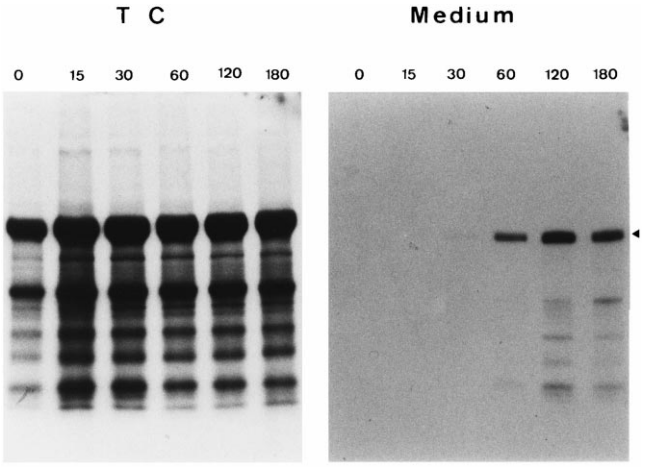

Fig. 3. Pulse-chase fluorography of teratocytes of *D. coccinellae* sampled from the host (*C. septempunctata*) at a late stage of parasitism. Teratocytes sampled from day 11 after parasitization were used. Experimental procedure was the same as in Fig. 2. An arrow indicates 94 kDa TSP subunit.

protein appeared to be released into the incubation medium quickly, rather than being accumulated in the fat body.

3.3. Glycosylation and amino acid composition of TSP

The major subunit (94 kDa) of TSP was identified as a high mannose glycoprotein on the basis of the affinity to Con A and LCA (*Lens calinaris* A) in lectin blots as shown in Fig. 5. The amino acid composition of the 94 kDa TSP subunit was also analyzed as shown in Table 1. The profile was quite similar to that of a nutritive protein, e.g. vitellogenin of *Locusta migratoria* (Chinzei et al., 1981) except that glycine was greater in TSP and leucine was greater in vitellogenin of *L. migratoria*. Native TSP (540 kDa) appears to be a hexamer judging from the molecular weight of the subunit (94 kDa). High content of asparagine and glutamine

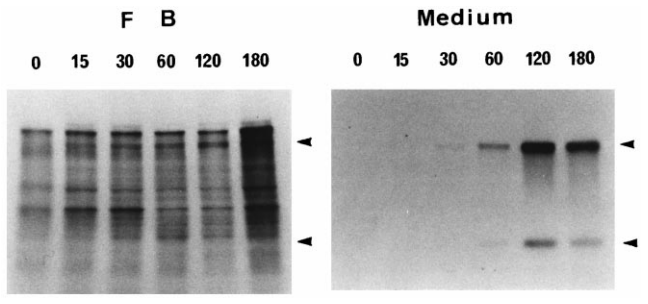

Fig. 4. Pulse-chase fluorography of fat body from non-parasitized host female. Fat body was sampled from an ovipositing female host. The procedure was as Fig. 2. Arrows indicate two vitellogenin subunits.

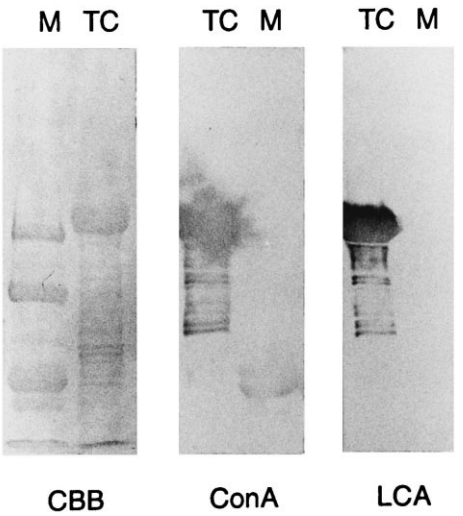

Fig. 5. Affinity blot of TSP against lectins. Teratocyte extracts were subjected to SDS-PAGE and followed by affinity blot against lectins. Lectins tested include, DBA (*Dolicoris biflours* A), Con A, LCA (*Lens calinaris* A), PNA (*Arachis hypogaea* A), UEA (*Ulex europaeus* A) and WGA (Wheat germ A). Only ConA and LCA had an affinity to TSP. TC: teratocyte extracts, M: marker proteins. Ovalbumin in the standard markers had an affinity to Con A.

characterized by insect storage hexamers (Telfer and Kenkel, 1991) are also found in TSP.

3.4. Localization of TSP in the teratocyte cells

To determine the distribution of TSP, ultra-thin sections of teratocytes from hosts on day 11 after parasitization were prepared for immunostaining with anti-TSP serum and second antibody conjugated with gold par-

Table 1
Amino acid composition of *D. coccinellae* 94 kDa teratocyte-specific polypeptide subunit

	n mol	%
Asp	3.2	10.2
Thr	1.3	4.2
Ser	3.3	10.7
Glu	3.9	12.5
Gly	4.2	13.5
Ala	2.0	6.5
Val	1.5	4.8
Cys	0.2	0.6
Met	0.5	1.5
Ile	1.1	3.4
Leu	2.0	6.3
Tyr	1.2	3.8
Phe	1.1	3.6
Lys	1.4	4.6
His	0.7	2.2
Arg	1.1	3.5
Pro	2.3	7.3

Proteins were hydrolyzed in 6 M HCL at 110°C in vacuo for 24 h and the amino acids were determined with a Hitachi model, L-8500.

ticles and followed by electron microscopy (EM). The gold particles, which indicated the presence of TSP, were specifically located in high electron-dense vacuoles (Fig. 6a and b). In sections treated with a preimmunized control serum, no gold particles were seen in the high electron dense vacuoles (Fig. 6c).

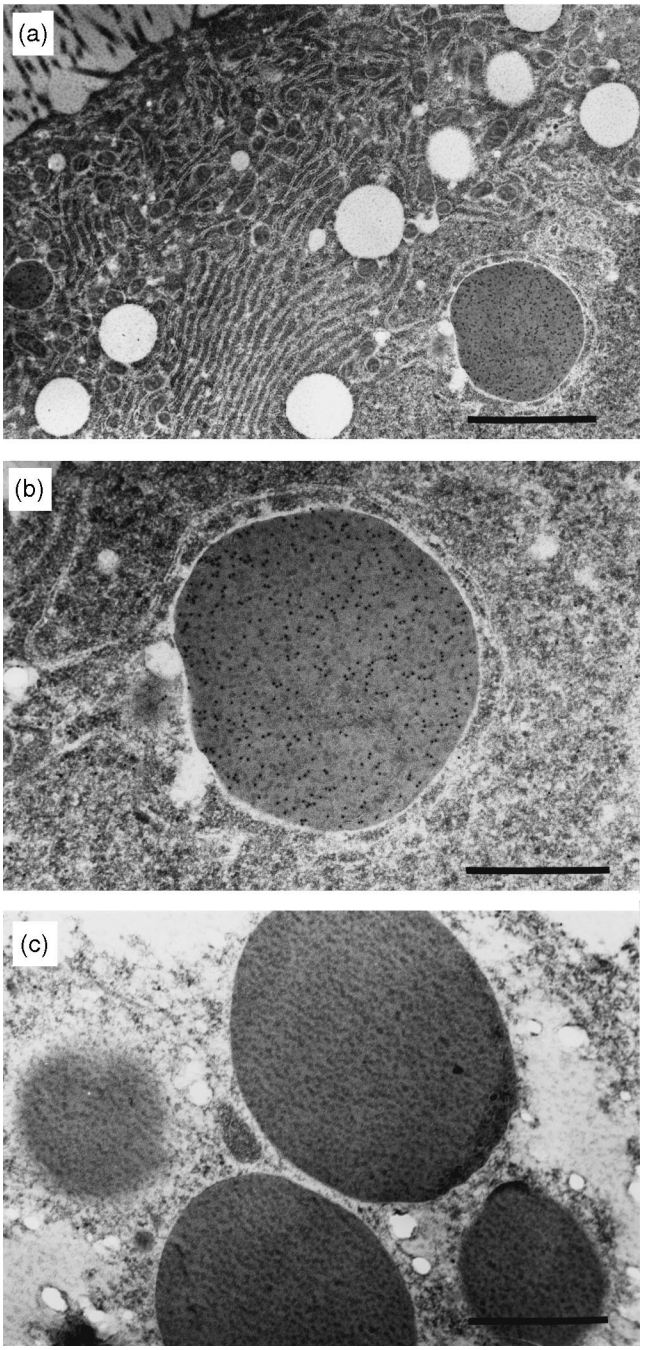

Fig. 6. Immunoelectronmicroscopic analysis of teratocytes. a: teratocytes of *D. coccinellae* from a host 8 days after parasitization prepared for ultra-thin sections, which were then treated with anti-TSP antibody and secondary antibody conjugated with gold particles. TSP was accumulated specifically in a high dense body. Reference bar represents 2 μm. b: magnified high density bodies treated with anti-TSP serum. Reference bar represents 1 μm. c: control staining of high density bodies treated with a preimmunized serum. Reference bar represents 1 μm.

Fig. 7 shows a parasitized host observed at day 11 after parasitization. Teratocytes, being spherical at an early stage, assumed an irregular shape and hypertrophied in the host body cavity. Some granules came out from the teratocytes in a form of exocytosis, and this was frequently observed after the teratocytes underwent hypertrophy (Fig. 7 arrows).

3.5. Determination of esterase activity in the extracts from teratocytes, host tissues and host hemolymph

Crude extracts from teratocytes, the host tissues and host hemolymph were separately subjected to native-PAGE, and esterase activity was detected with the substrate α-naphtyl acetate. The esterase zymogram indicated that teratocytes had a band corresponding to 540 kDa with esterase activity (Fig. 8). This band was cut, eluted, and then subjected to SDS-PAGE followed by CBB staining, but the electropherogram showed only a single band with a molecular weight of 94 kDa (Data not shown). This result suggests that the 94 kDa subunit is responsible for the esterase activity. In the zymograms for the host hemolymph, a positive band corresponding to 540 kDa TSP was found only in the parasitized host hemolymph (Fig. 9), which also supported the conclusion that TSP has esterase activity.

3.6. Determination of juvenile hormone esterase activity in the extracts from teratocytes

General esterase activity exhibited by a crude teratocyte extract was confirmed with a microplate enzyme assay using the substrate p-nitrophenyl acetate

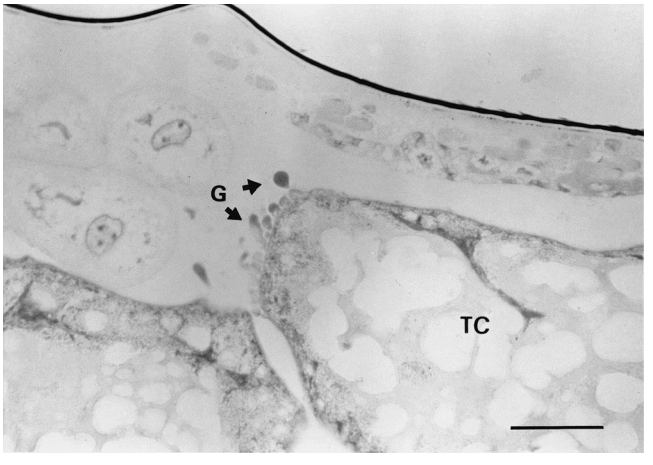

Fig. 7. Hypertrophic teratocytes at late parasitism in the host body cavity. The whole body of a *C. septempunctata* host at day 11 after parasitization was embedded in paraffin and sectioned, followed by Delafield's hematoxylin and eosin staining. An indication of granule eruption from hypertrophied teratocytes is evident. G: granule, TC: teratocyte. Reference bar represents 30 μm.

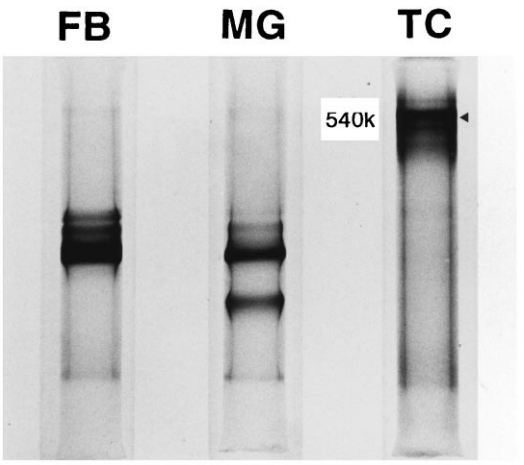

Fig. 8. Esterase zymogram of extracts from *C. septempunctata* host fat body, host midgut and teratocytes of *D. coccinellae*. FB: extracts from host fat body, MG: extracts from host midgut, TC: extracts from teratocytes. Each extract sample was subjected to native-PAGE (7.5%) and followed by esterase zymography. A positive band (arrow) with high molecular weight corresponds to 540 kDa TSP.

(enzymatic activity = 311 nmol/min/mg) (Fig. 10B and C), but the teratocyte extract showed no juvenile hormone esterase activity (enzymatic activity = 13.7 nmol/min/mg), indicating that TSP is not responsible for hydrolyzing juvenile hormone (Fig. 10D and E).

4. Discussion

Teratocytes of *Dinocampus* may play primarily a role in providing nutrients to the growing parasitoid larva in the hosts (Sluss, 1968; Kadono-Okuda et al., 1995; Okuda and Kadono-Okuda, 1995). This assumption is supported by the present findings that the TSP major subunit (94 kDa), identified as a glycoprotein with high amount of mannose, was similar in the amino acid composition to vitellogenin of *L. migratoria* (Chinzei et al., 1981) and other insect storage hexamers (Telfer and Kenkel, 1991) (Table 1). The reason why teratocytes of this parasitoid actively synthesize and store such a vitellogenin-like nutritive protein and become hypertrophic may be related to the fact that larvae and adults store nutrients in different ways. In general, insect larvae store protein reserves in the hemolymph as storage proteins (Tojo et al., 1980). Parasitoid larvae present in the hemolymph of the larval host should have easy access to protein nutrients. However, adult hosts have far less storage protein in the hemolymph compared with larval host, and so parasitoids in adult hosts have to rely on newly absorbed protein nutrients from feeding by the adult hosts. The fat body is an important tissue for storage of nutritive reserves for adults. In adulthood flight is an important means of food-searching, and during flight, fat body functions as a generator of fuel (de Kort, 1969).

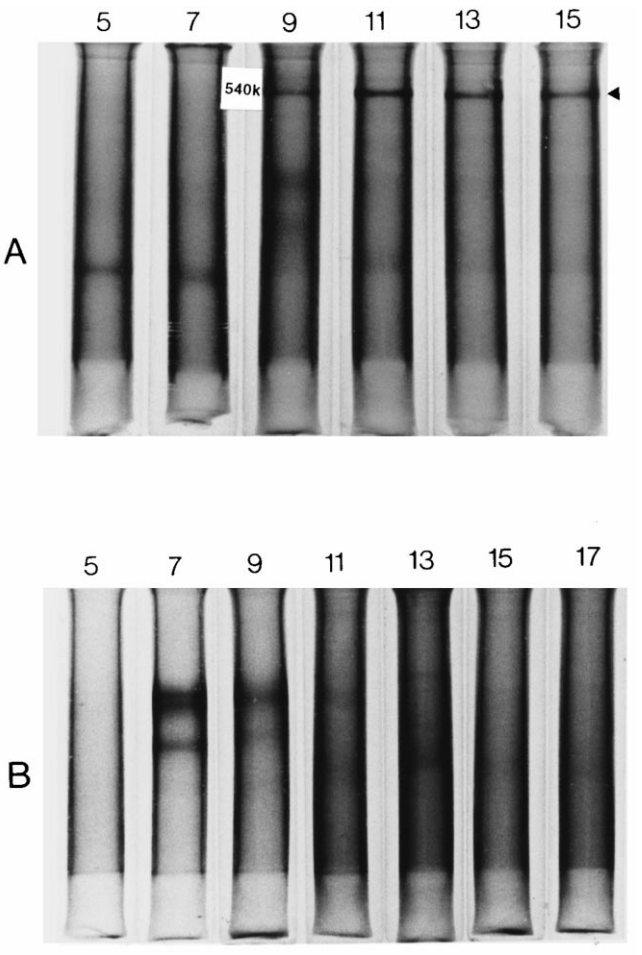

Fig. 9. Esterase zymogram of hemolymph from parasitized and non-parasitized *C. septempunctata* hosts. A: parasitized host hemolymph. A positive band (arrow) with high molecular weight corresponds to 540 kDa TSP. B: non-parasitized host hemolymph. The number above each gel indicates the number of days after parasitization. Procedure for zymography was the same as Fig. 8.

Therefore eating host fat body may not be a good strategy for parasitoid larvae. This idea is in agreement with other reports on parasitoids of adult stage host, i.e. teratocytes of braconid wasps, *Microctonus aethiopoides* and *Dinocampus rutilus* in the adult hosts, *Sitona discoideus* (Barratt, personal communication) and *Sitona lineata* (Jackson, 1928) respectively show similar changes in size and number. Alternatively, since coccinellids possess defensive compounds against natural enemies (Tursch et al., 1976; Eisner et al., 1986) teratocytes might provide a source of safe food for parasitoids. Coccinelline, an alkaloid which has a very bitter taste, amounts to 1.5% of the dry weight of *C. septempunctata* and its protective function against predators has been demonstrated (Pasteels et al., 1973). It is possible that the alkaloid is also toxic to the parasitoid larva, a possibility that is currently being tested. The teratocytes appear to contain little or no alkaloid (unpublished data). If larvae of *D. coccinellae* could depend completely on

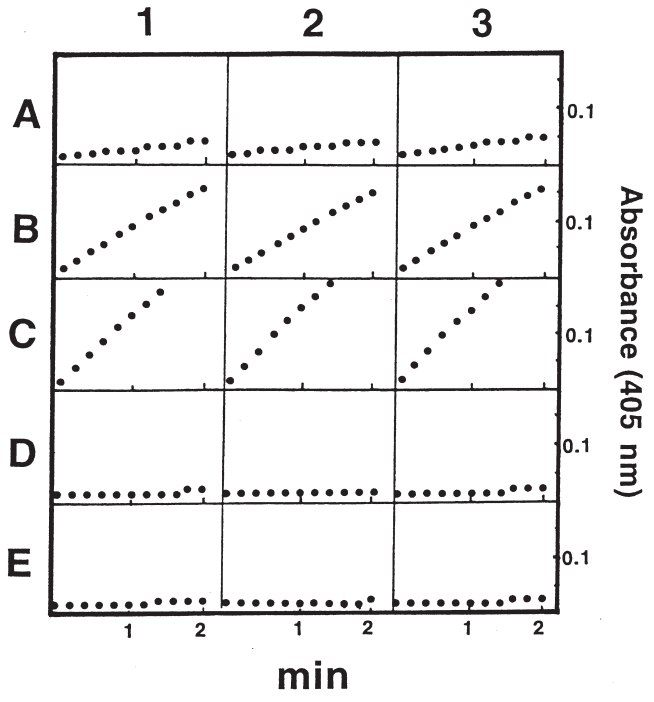

Fig. 10. Quantitative kinetic assays for general esterase and JH esterase in crude teratocyte extracts. A: substrate (p-NPA) only for control, B: 2.5 μl teratocyte extracts and substrate (p-NPA), C: 5 μl teratocyte extracts and substrate (p-NPA), D: 2.5 μl teratocyte extracts and substrate (HEPTAT), E: 5 μl teratocyte extracts and substrate (HEPTAT). Each assay was repeated 3 times.

teratocytes, as observed by Ogloblin (1924), they may be able to avoid alkaloid in the host. If this is the case, teratocytes should synthesize a protein like vitellogenin which contain an ideal amino acid composition for the parasitoid larvae to grow.

So far, hypertrophic teratocytes have not been found in gregarious parasitoids. In solitary parasitoids the hypertrophic nature of teratocytes may not be a common feature because teratocytes in some solitary parasitoids of larval stage hosts, *Chelonus inanitus* (Grossniklaus-Burgin et al., 1994) and *Telenomus heliothidis* (Strand et al., 1986) do not become hypertrophic. More information from many other species is needed in order to generalize.

The accumulation of TSP in *D. coccinellae*, as indicated by a pulse-chase analysis (Figs. 2 and 3), suggests that teratocytes are significant in providing nutrients to parasitoid larvae in an efficient manner. Teratocytes store TSP gradually without causing deterioration in host viability, and they are mostly ingested during the late stage of parasitism when parasitoid larvae attain the second instar larvae and grow rapidly (Okuda and Kadono-Okuda, 1995). The accumulation of TSP appears unique in that other nutritive proteins such as vitellogenin and storage proteins are usually released immediately after synthesis (Engelmann, 1970), as we confirmed for the fat body of *C. septempunctata* (Fig. 4). It is probable that the protein release mechanism may differ between vitellogenin or storage proteins, and TSP. Release of the former proteins is mediated by signal peptides (Loche et al., 1987; Trewitt et al., 1992), while the later may be released through exocytosis (Fig. 7), though further evidence is required.

Immunohistochemical studies by EM indicated that the TSP of *D. coccinellae* was specifically located in a vacuole in the teratocytes (Fig. 6). These vacuoles are referred to as electron dense bodies (Sluss, 1968; Sluss and Leutenegger, 1968). It has been speculated that these dense bodies are pools of nutrients absorbed from the host hemolymph. However, the present results together with previous data (Okuda and Kadono-Okuda, 1995) strongly suggested that these dense bodies are pools of TSP synthesized by teratocytes themselves.

Strand et al. (1986) demonstrated that the cultured media with teratocytes of an egg parasitoid, *Telemonus heliothidis* had enzymatic activities of acid phosphatase, esterase and leucine aminopeptidase. This was the first direct evidence that teratocytes produce enzymes. These enzymes are assumed to be responsible for host tissue break-down. In the present study, we also found that TSP of *D. coccinellae* has esterase activity (Figs. 8–10). As TSP mostly remains in the teratocytes and only a slight amount moves out into the host hemolymph (Figs. 1 and 2), it is unlikely that TSP functions as a lysis enzyme to break-down the host tissues. Parasitism often induces host castration (Beckage, 1985). When *C. septempunctata* female adults were parasitized by *D. coccinellae*, vitellogenesis by the host fat body was strongly inhibited (Fig. 1). It is possible that the titer of juvenile hormone, which promotes vitellogenesis, was lowered somehow by the parasitism. We then investigated whether TSP is able to degrade juvenile hormone (Fig. 10). The results were negative, the biological role of TSP as an esterase is thus yet to be solved.

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