Inhibition of lipid transport in insects by a factor secreted by the parasite, *Blepharipa sericariae*

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A parasitoid fly secretes a factor into hemolymph of the host silkworm, which inhibits lipid transport from the host's fat body by lipophorin. The available data indicate that the factor is a small peptide with a molecular mass of less than 3500 Da.

Parasitoid *Lipoprotein* *Lipophorin* *Lipid transport* *Peptide secretion* *Transport inhibitor*

1. INTRODUCTION

The relation of insect parasitoids with their hosts has been investigated from the viewpoints of defence mechanism [1], endocrinology [2] and metabolism [3,4]. Most of these studies have focussed on the response of the host to the parasitoid, whereas few attempts have been made to investigate physiological actions that occur within the parasitoid.

Lipophorin, a major lipoprotein of insect hemolymph, serves as a re-usable shuttle to transport various lipids, including diacylglycerol, hydrocarbons and cholesterol from sites of storage, absorption or synthesis to sites of utilization or deposition [5]. More than 50% of dormant pupae of the silkworm, *Philosamia synthia*, collected from the field in fall are parasitized by larvae of the fly, *Blepharipa sericariae*. The larvae remain in the second instar until the following spring when they begin to feed on the host tissue eventually causing the death of the pupa. The wax layer of parasitized pupae is considerably thinner than that of unparasitized pupae thus implying that the lipid-transporting and/or lipid-mobilisation systems of the host may be impaired by the parasitoid. The present study was designed to test the possibility that lipid release from the fat body and/or uptake by lipophorin are perturbed by the parasitic larvae. This paper reports that the parasitic larvae secrete a small peptide into host hemolymph, which retards the transport of diacylglycerol from the host's fat body, the major site of intermediary metabolism in insects and an important storage organ for nutrient reserves.

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2. EXPERIMENTAL

Hemolymph, freshly collected from parasitized and unparasitized dormant pupae of *P. synthia*, was immediately mixed with phenylthiourea (final concentration, 0.5 mM) and centrifuged at 8000 × *g* for 5 min at 4°C to remove the hemocytes. The supernatant was used as a hemolymph preparation. Lipophorin was purified from hemolymph of unparasitized silkworm pupae according to Chino et al. [6].

The dissected and rinsed fat body from unparasitized dormant pupae was prelabelled by incubating with [1-^{14}C]palmitic acid (approx. 1 × 10^{-7} Ci/100 mg fat body) for 90 min. After labelling, the fat body was thoroughly washed with physiological saline (120 mM NaCl, 10 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 20 mM Pipes buffer, pH 7.0). The 'prelabelled' fat body thus prepared, contains [^{14}C]triacylglycerol and [^{14}C]diacylglycerol.

The capacity of hemolymph, or purified lipophorin, to take up lipid (mainly diacylglycerol)

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from the fat body was determined by incubating the prelabelled fat body in physiological saline containing hemolymph (24 mg/ml protein for each incubation) or purified lipophorin (0.4 mg/ml for each incubation) at 30°C for 90 min. The final incubation volume was 0.5 ml and 50–80 mg prelabelled fat body was used for each incubation. After incubation, lipids were extracted from the incubation medium with isopropanol:heptane (4:1) [7], and the radioactivity counted on a liquid-scintillation counter. In some experiments, the extracted lipids were separated to each lipid class on TLC (solvent system: hexane/diethyl ether/acetic acid, 70:30:1, v/v) to determine the class of lipid that is affected by the parasitic factor. The results of these determinations confirmed that the percentage decrease of total lipid was equivalent to that of the diacylglycerol fraction.

For each proteolytic enzyme treatment, 20 μg/ml proteolytic enzyme was added to the preparation containing parasite factor and each mixture was incubated for 6 h at 30°C. After incubation the reaction was stopped by heating in a boiling water bath for 3 min.

For high-performance liquid chromatography of the active fraction obtained from Bio-Gel P-6 column gel filtration, a column (7.5 × 750 mm) of DEAE-5PW (Toyo Soda, Japan) was used. A linear gradient elution of 0–30% NaCl in 20 mM Tris-HCl buffer (pH 8.2) containing 10% acetonitrile was performed at a flow rate of 1.0 ml/min. Each fraction corresponding to peaks manually collected was concentrated to remove acetonitrile by a nitrogen stream, and the remaining aqueous solution was added to the assay system for lipid uptake from the prelabelled fat body by lipophorin.

The amino acid composition of purified parasitic factor was determined on a Hitachi 835 automatic analyzer following hydrolysis in 4 M methanesulfonic acid at 110°C for 24 h.

3. RESULTS AND DISCUSSION

The capacity of parasitized hemolymph to accept lipid from the fat body is about one-half that of unparasitized hemolymph (fig.1). The lipid uptake from the prelabelled fat body by lipophorin from unparasitized pupal hemolymph was reduced by 50–70% when intact larvae were added to the lipid uptake assay system (not shown). These results suggest that the parasitoids secrete a factor which inhibits lipid uptake from the fat body by lipophorin. This possibility was tested by determining if an incubation medium that has been incubated previously with intact parasitoid larvae, inhibits lipid uptake by purified lipophorin. About 30 larvae were incubated with 10 ml physiological saline for several hours at 30°C, and 0.1 ml of this pre-incubation medium was added to the assay system (total 0.5 ml). Lipid uptake was considerably inhibited (maximal inhibition about 60%) by addition of the pre-incubation medium, thus indicating that the intact parasitoids secrete a factor into the saline, which inhibits lipid transport from the fat body by lipophorin. An estimation of the molecular mass of the factor was obtained by dialyzing 3 ml physiological saline containing 30 larvae in a special dialyzing tube (Spectrum USA; compounds of molecular mass greater than 3500 Da are not dialysable) against 7 ml physiological saline for 10 h at 30°C. After dialysis, the dialysable solution was tested for its ability to affect lipid uptake from the fat body by lipophorin. As illustrated in fig.2, the extent of lipid uptake steadily decreases as increasing amounts of the dialysable solution are added to the assay system. This indicates that the molecular mass of the factor secreted from the intact

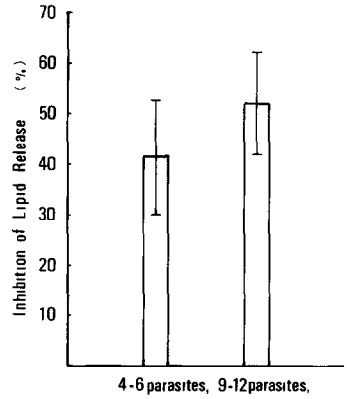

Fig.1. Inhibition of lipid release from the prelabelled fat body observed in parasitized hemolymph. A, hemolymph collected from pupae parasitized with 4–6 larvae (n = 10); B, hemolymph collected from pupae containing 9–12 larvae (n = 6). The inhibition rates are expressed as the percentages of lipid released into parasitized hemolymph against that into unparasitized hemolymph. The major class of released lipid was diacylglycerol. Vertical bars indicate ± SD.

parasitoid is smaller than about 3500 Da. The dialysable solution was then centrifuged at 200000 × g for 3 h, and the supernatant concentrated under a stream of nitrogen. The concentrated supernatant (2 ml) was applied to a Bio-Gel P-6 column (38 × 1.5 cm). After elution with 20 mM Tris-HCl buffer (pH 8.2), the peak fraction containing biological activity was collected (recovery: about 70%) and an aliquot subjected to HPLC separation. A single primary peak was observed at a retention time of about 19 min (fig.3) and the biological activity (20–30% inhibition) was located only at the main peak. The peptidergic nature of the active fraction was confirmed by demonstrating that biological activity was lost following treatment with pronase (table 1). The main peak on HPLC was then subjected to amino acid analysis and the results indicate that the peptide is rich in glycine but contains no tryptophan.

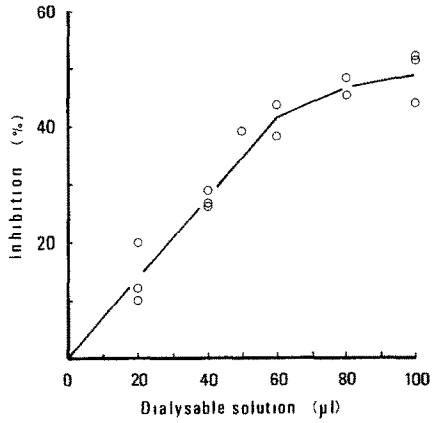

Fig.2. Effect of the dialysable factor on lipid uptake by lipophorin from the fat body. Abscissa, amount of the dialysable solution (μl) added to the incubation mixture.

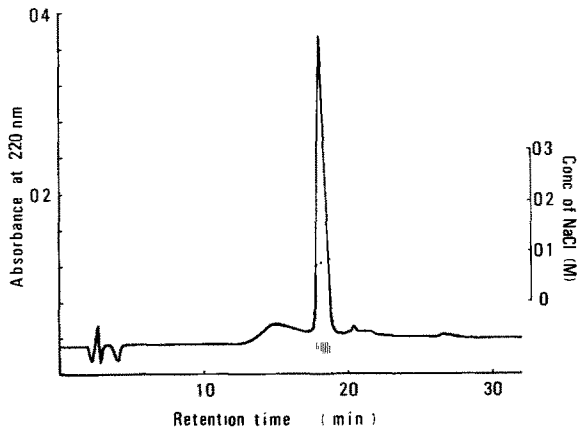

Fig.3. High-performance liquid chromatography of the active fraction obtained from Bio-Gel P-6 column chromatography. (——) Absorbance at 220 nm, (– – –) concentration of NaCl (M), (|||||) active fraction.

Table 1

Effect of some kinds of protease against parasitoid factor

Proteolytic enzyme	Inhibition (%) of lipid uptake		Control/ treated
	Control	Treated	
Pronase	80	99	0.81
Thermolysin	78	77	1.0
Papain	79	79	1.0
Aminopeptidase M	80	79	1.0

Each value represents the mean of duplicate determinations

The possible biological significance of this factor may be to conserve the stored lipids (mainly triacylglycerol) of the host's fat body for the parasitic fly to utilize as an energy source until the following spring. Further characterization of this new peptide is now in progress.

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