Structure of a Growth-blocking Peptide Present in Parasitized Insect Hemolymph*

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Last instar larvae of the insect armyworm, Pseudaelia separata, parasitized with the parasitoid wasp, Apanteles kariyai, do not initiate metamorphosis and, ultimately, the wasp larvae emerge from the host larvae about 10 days after parasitization (Tanaka, T., Agui, N., and Hiruma, K. (1987) Gen. Comp. Endocrinol. 67, 364–374). It is necessary for the parasitoid wasp to perturb the armyworm’s endocrinological processes that control normal metamorphosis from larvae to pupae. This endocrinological perturbation allows the parasitoid to complete its larval growth before emerging from the host larvae. It is obligatory for the parasitoid larvae to emerge while the host is still in a larval stage because the sclerotized pupal cuticle is impenetrable for the parasitoid larvae. A growth-blocking peptide with repressive activity against juvenile hormone esterase has been proven to exist in the parasitized host larval plasma (Hayakawa, Y. (1990) J. Biol. Chem. 265, 10813–10816). Here, I describe the detailed structure of this peptide and also the corresponding synthetic peptide to confirm this structure.

Insect endoparasitoids employ a variety of mechanisms to suppress the endocrine system to disrupt metamorphosis of holometabolous host insect (3, 4). Although many studies have focused on phenomena such as deficient ecysteroid hormone production (5–8), lack of synthesis of prothoracicotropic hormone (9), and modifications of JH1 levels (10, 11), the biochemical explanation for such endocrine alterations in host insects has not been given. JH esterase activity repressive peptide found in the parasitized armyworm larval plasma (hemolymph devoid of cells) is the first reported natural factor that is responsible for the interruption of metamorphosis (2). The mechanism by which JH esterase activity repressive peptide causes a delay in the onset of pupation has not been completely elucidated, but we presume it functions as follows. In the last larval instar of holometabolous insect, JH controls a cascade of events leading to pupation; the rapid decline of JH concentration in plasma, initially high at the beginning of the last instar, is essential to the release of prothoracicotropic hormone acting on the prothoracic glands to stimulate the release of ecysteroid hormone (12–15). Because JH esterase in larval plasma accounts for the major hydrolysis of JH (16, 17), the plasma of the last instar larvae whose JH esterase activity is repressed by the growth-blocking peptide should maintain high JH levels, thus blocking the host larval development. It can be said that JH esterase is a key enzyme for controlling insect metamorphosis because this enzyme is one of the first components which is responsible for initiating a normal metamorphosis from larvae to pupae (18–20). In this respect, more detailed investigations concerning the nature of this peptide were warranted.

I present herein the complete structure of the growth-blocking peptide whose existence and purification have been reported in the previous paper (2).

MATERIALS AND METHODS

Animals—Pseudaelia separata were reared on an artificial diet at 25 ± 1 °C with a photoperiod of 16 h light:8 h dark (1). Parasitization by Apanteles kariyai was carried out by exposing prospective hosts (day 0 last instar larvae) to female wasps. After a single oviposition was observed, the parasitized larvae were quickly removed in order to avoid superparasitization and then reared on the artificial diet. Adult wasps were maintained with honey.

Chemicals—[10-3H]JH I (15.5 Ci/mmol) was purchased from Du Pont. Peptides were synthesized by solid phase procedures using a Biolyx 4170 automated peptide synthesizer (Pharmacia LKB Biotechnology Inc.).

Bioassay and Enzyme Assay of Peptide—The purified or synthetic peptide was diluted with Ringer’s solution to the desired concentration (final volume, 5 μl) and injected into unparasitized last instar larvae once at the indicated day(s). Controls were injected with 5 μl of Ringer’s solution only. The next day after the last injection, plasma was prepared from the larvae for assay of JH esterase activity.

Plasma degradation of [10-3H]JH I and unlabelled carrier hormone (E, E-cis-JH I) (Sigma) was measured in vitro using the methods of Hammock and Roe (21). Every enzyme preparation (plasma) was first preincubated with 2 × 10−4 M diisopropyl fluorophosphate (Sigma) at 25 °C for 10 min prior to addition of substrate (final concentration, 5 × 10−5 M).

Peptide Characterizations—The NH2-terminal amino acid sequences of peptides were analyzed by automated Edman degradation with a protein sequenator (model 477A, Applied Biosystems) (22). The sequences were verified by analyzing about 200 pmol of the peptides 3–4 times.

The measurement of fast atom bombardment mass spectrum was made using a JMS-HX 110 mass spectrometer (JEOL) fitted with a standard fast atom bombardment ion source. The source was operated with a 6-keV xenon atom, and glycerol was used as a matrix.

Amino acid analysis was achieved using a Hitachi 835 automatic analyzer following hydrolysis of the purified protein in 6 M HCl at 110 °C for 24 h.

RESULTS AND DISCUSSION

The JH esterase activity repressive peptide was isolated from parasitized armyworm larval plasma by a 6-step purification procedure involving extraction with 25% ethanol, gel filtration, and reversed phase high performance liquid chromatography (2). Analysis of the peptide after acid hydrolysis gave the amino acid composition as follows: Asx2—Glx2—Ser5—Glyx3—Argx2—Thr2—Alax2—Pro2—Tyr1—Val1—Met1—Phe2—Lys2—Cysx2. The NH2-terminal amino acid sequence of the intact peptide was determined by automated Edman degradation as shown in Fig. 1A. The analysis of carboxymethylated or pyridylethylated peptide gave the NH2-terminal amino acid sequence as shown in Fig. 1B. Furthermore, the analysis of the trypsinized
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Asn-Phe-Se-Gly-Gly-Val-Ala-Gly-Tyr-Met-Arg-Thr-Pro

Asp-Gly-Arg-Val-Ala-Gly-Tyr-Met-Arg-Thr-Pro

Asp-Gly-Cys-Lys-Pro-Thr-Tyr-Gln-Oh

FIG. 1. A, amino-terminal amino acid sequence of intact JH esterase activity repressive peptide; B, amino acid sequence of the derivatized peptide; C, the structure of the peptide with amino carboxyl-terminal group. The amino acid sequence was analyzed by automated Edman degradation with a protein sequenator (model 477A, Applied Biosystems).

Fig. 2. A, fast atom bombardment mass spectrum of the intact peptide; B, theoretical ion distribution calculated for the peptide amino acid sequence shown in Fig. 1B.

FIG. 3. Reversed phase HPLC on a large-pore C18 column. A, mixture of amino carboxyl-terminal synthetic peptide and purified peptide chromatographed with CH3CN in 0.1% CF3COOH/H2O; B, mixture of free carboxyl-terminal synthetic peptide and purified peptide chromatographed with CH3CN in 0.1% CF3COOH/H2O.

Pseudaletia separata

Day 2 last instar larvae

CONTROL

TREATED

FIG. 4. The effect of JH esterase repressive peptide on insect. Middle, Day 2 last instar larvae injected with 7 pmol of the synthetic peptide amide (Fig. 1C) at Day 0 and 1, respectively; right, Day 2 larvae injected with 3 pmol of the free carboxyl terminus once at Day 0; left, control larvae injected with Ringer's solution at Days 0 and 1, respectively.

FIG. 5. The weight and timing of pupation of the peptide-injected insect. Last instar larvae were injected with 7 pmol of the purified (×) (n = 15) or the synthetic peptide (○) (n = 11) for 2 consecutive days (Day 0 to Day 1). Control larvae were injected with only Ringer's solution (●) (n = 9). The number in parentheses indicates the percentage of prepupated insects at each day. At the time of prepupation, weight decreases as shown by the dotted line. Each point represents mean ± S.D. for the number of determinations indicated in parentheses. Purified peptide-treated values are not significantly different from synthetic peptide-treated values, but these peptide-treated values are significantly different from control values after Day 1 (p < 0.001).
armyworm. The mixture of the purified peptide and the synthetic peptide was chromatographed by a reversed phase C18 HPLC column (Yamamura Co., Japan; 4.6 mm (inner diameter) × 250 mm, pore size = 300 Å) using gradient elution from 1 to 40% CH3CN in 0.1% CF3COOH/H2O at a flow rate of 0.4 ml/min (Fig. 3). The amino carboxyl-terminal peptide was coeluted with the purified peptide at exactly the same retention time (Fig. 3A), but the free carboxyl-terminal peptide was eluted about 1.0 min later than the purified peptide under these conditions (Fig. 3B). These chromatographic patterns indicate that the amino carboxyl-terminal peptide and purified peptide are the same. In order to confirm this physiologically, the following experiments were carried out. Day 2 last instar larvae injected with 7 pmol of the synthetic peptide amide on Days 0 and 1, respectively, are significantly smaller (p < 0.0001) than the control larvae which were injected with Ringer's solution as shown in Fig. 4. Furthermore, the larvae treated with the peptide amide have only 10% of plasma JH esterase activity to that of the controls and showed no mortality. However, all of the larvae given only one injection of 3 pmol of the free carboxyl-terminal synthetic peptide (Fig. 1B) on Day 0 died within 48 h after the treatment (Fig. 4). These results strongly suggested that the synthetic peptide with an amino carboxyl-terminal group is truly the same as the purified JH esterase repressive peptide. The free carboxyl-terminal peptide, on the contrary, shows a strong insecticidal effect with a median lethal dosage (LD50) of 2 pmol/larva (average weight of tested Day 0 last instar larvae: 0.1929 ± 0.0158 g (mean ± S.D., n = 38)). Fig. 5 shows the measurements of the weight gain and the timing of pupation of last instar larvae which were injected with 8 pmol of the purified peptide and the synthetic peptide amide on Days 0 and 1, respectively. The application of the both peptides (purified and synthetic) clearly retards the larval growth and, consequently, causes more than a 2-day delay in pupation. The results of these experiments confirm that the pentacosane peptide amide containing one disulfide bond shown in Fig. 1C is the complete structure of the growth-blocking peptide present in parasitized armyworm larval plasma. Although the current study does not explain the mechanism by which this peptide blocks the larval growth, it is possible that it is expressed through the disturbance of neuroendocrinological processes. JH esterase activity may be repressed indirectly after the growth-blocking peptide interferes with initial steps in a cascade of events leading to normal metamorphosis.

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