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Juvenile hormone controls early trypsin gene transcription in the midgut of *Aedes aegypti*

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Abstract

Early trypsin is a female-specific protease present in the *Aedes aegypti* midgut during the first few hours after ingestion of a blood meal. The enzymatic activity of early trypsin plays an essential role in the transcriptional activation of the late trypsin gene, which encodes the major midgut endoprotease involved in blood meal protein digestion. Transcription of the early trypsin gene is part of the normal post-emergence maturation of the midgut in the adult female. Abdominal ligation within 1 h of emergence completely prevented the transcription of the early trypsin gene. Topically applied JH III or methoprene induced transcription of the early trypsin gene in ligated abdomens to levels similar to those observed in non-ligated females. The induction of early trypsin transcription by JH is dose-dependent and 'head-independent', suggesting that factors coming from the neurosecretory axis are not required.

Keywords: mosquito, trypsin, juvenile hormone.

Introduction

Juvenile hormone (JH) regulates several aspects of reproductive maturation in the adult female mosquito, including previtellogenic oocyte growth, fat body 'competence', proliferation of ribosomes in the fat body and development of the follicle cells (recently reviewed by Hagedorn, 1994). The JH level increases during the first 2 days after adult emergence from two pg/female to 20 pg/female. When a female takes a blood meal, the JH level falls rapidly during the first 3 h to 6 pg/female, and then the rate of decline slows, such that the level reaches its lowest point (1.5 pg/female) 24 h after the blood meal. 48 h after the blood meal the JH level starts to rise again, and after 96 h it is equivalent to the pre-blood meal value (Shapiro *et al*., 1986).

Early trypsin is a female-specific protease present in the *Aedes aegypti* midgut during the first few hours after ingestion of a blood meal (Noriega *et al*., 1996a). Its enzymatic activity is essential for normal blood meal digestion (Barillas-Mury *et al*., 1995). The early trypsin mRNA, absent in larvae, pupae and newly emerged females, reaches detectable levels during the first 24 h after adult emergence and attains a maximum level at an adult age of 3–7 days. Despite the high level of early trypsin mRNA present in the midgut of the unfed female, translation of the early trypsin mRNA occurs only after ingestion of a blood meal or a protein meal (Noriega *et al*., 1996b). Early trypsin mRNA levels decrease rapidly during the first 24 h after feeding and the steady-state level of the transcript rises again at the end of the blood digestion cycle (60 h) (Noriega *et al*., 1996b).

The developmental pattern of the expression of the early trypsin gene is very similar to the pattern of the developmental changes of the JH level in females, suggesting that JH might be involved in regulating early trypsin gene expression. In this paper we describe experiments on the role of JH in the regulation of early trypsin gene expression.

Results

Effect of abdominal ligation on expression of the early trypsin gene

Northern blot analysis of early trypsin mRNA levels in unligated females during the first 24 h after adult emergence (Fig. 1), showed that the early trypsin mRNA was absent in newly emerged females, and that the transcript level increased dramatically after 10 h. Abdominal ligation within 1 h of adult emergence reduced the transcription of the early trypsin gene below detectable levels (Fig. 2). When the ligations were performed 6 or 8 h after adult emergence, the levels of early trypsin mRNA, measured 24 h after emergence, were comparable to non-ligated females (Fig. 3).

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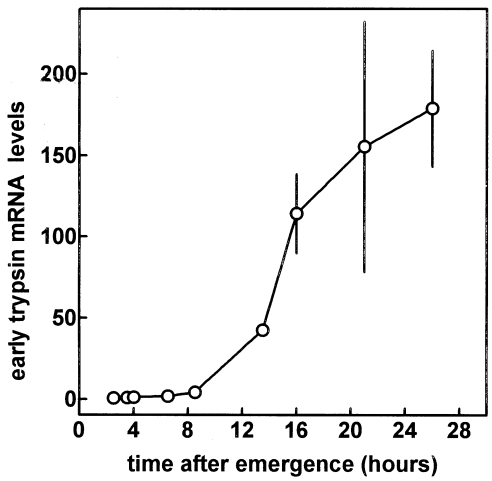

Figure 1. Early trypsin mRNA levels following adult emergence. Each point represents the mean ± SD of duplicate analyses of groups of five mosquitoes. One mosquito/equivalent was loaded in each lane. By staining the gel with ethidium bromide we confirmed that the amounts loaded were equivalent. Early trypsin mRNA levels are cpm of early trypsin mRNA/insect after background subtraction.

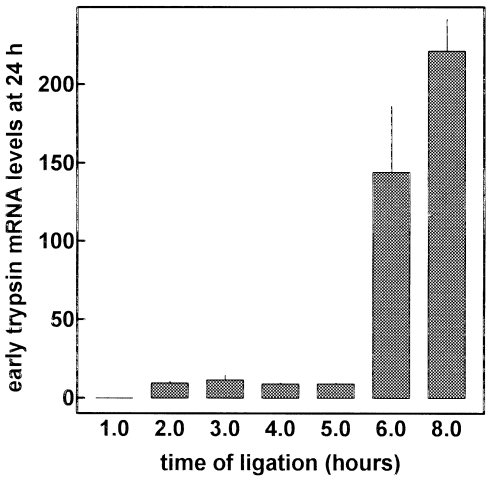

Figure 3. Effect of the time of ligation on early trypsin mRNA levels. Each sample represents means ± SD of duplicate analyses of groups of five abdomens. Early trypsin mRNA levels are cpm of early trypsin/five abdomens after background subtraction. Times of ligation are hours after adult emergence.

Effect of topically applied methoprene and JH III on early trypsin gene expression in ligated abdomens

The finding that abdominal ligation could silence early trypsin gene transcription prompted us to investigate whether JH could restore the expression. Topically applied methoprene (500 pg/abdomen) induced significant transcription of the early trypsin gene in ligated abdomens (Fig. 2). The values of early trypsin mRNA in ligated abdomens were 70% [n = eighteen groups of five abdomens each] of the values of early trypsin mRNA in non-ligated abdomens. Methoprene applied immediately after emergence accelerated the expression of the early trypsin gene when compared with controls (Fig. 4).

A dose–response effect was evident when using both methoprene and JH III (Fig. 5). 100 pg of methoprene per insect induced high expression of the early trypsin gene, whereas 100 ng of JH III per insect was necessary to elicit a similar increase in early trypsin gene expression.

Effect of methoprene on the expression of the early trypsin gene after feeding

Females were treated topically on the abdomen with methoprene (10 ng/insect) or acetone within 1 h after receiving a protein meal. Early trypsin mRNA levels in both methoprene-treated females and controls, decreased during the first 4–6 h. However, 12 h after

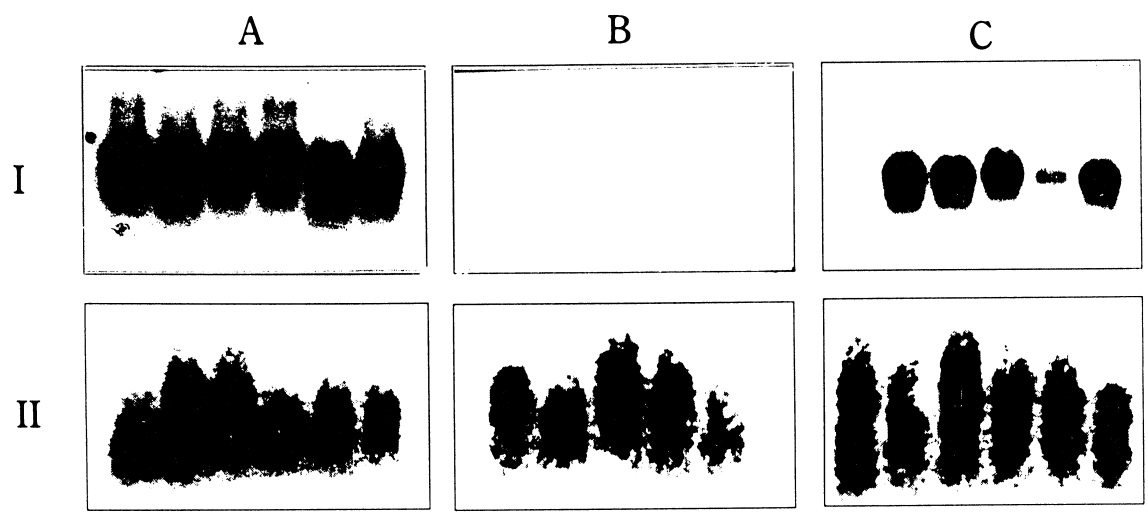

Figure 2. Effect of abdominal ligation on early trypsin mRNA levels. Northern blot hybridized with: (I) an early trypsin probe; (II) a ribosomal probe. (A) Controls (abdomens from non-ligated females). (B) Abdomens (ligated 1 h after adult emergence) topically-applied with 0.5 μl of acetone. (C) Abdomens (ligated 1 h after adult emergence) topically-applied with methoprene (500 pg/abdomen). Each sample represents total RNA from five abdomens, 24 h after adult emergence.

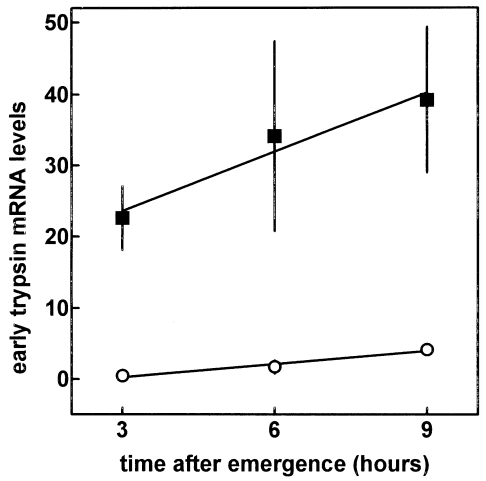

Figure 4. Effect of methoprene on early trypsin mRNA levels in newly emerged adults. Females were topically applied with methoprene (500 pg) or acetone (0.5 μl) within 1 h of adult emergence. Each sample represents means $\pm$SD of triplicate analyses of groups of five abdomens. Early trypsin mRNA levels were calculated as described in Fig. 3. The lines are linear regression lines. ■: methoprene; ○: acetone.

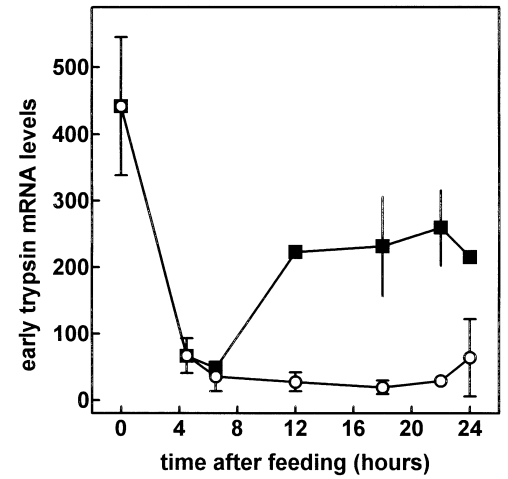

Figure 6. Effect of methoprene on expression of early trypsin after feeding. Each sample represents means $\pm$SD of duplicate analyses of groups of five mosquitoes. Early trypsin mRNA levels were calculated as described in Fig. 1. ○: Females topically applied with acetone; ■: females topically applied with methoprene (10 ng/insect).

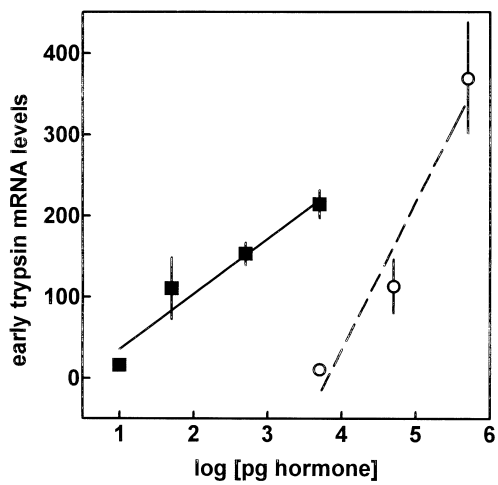

Figure 5. Dose–response curves. Each sample represents means $\pm$SD of triplicate analyses of groups of five abdomens. Early trypsin mRNA levels were calculated as described in Fig. 3. The lines are linear regression lines. ■: methoprene; ○: juvenile hormone III.

feeding, the transcript levels in methoprene-treated females increased and were higher than acetone-treated controls (Fig. 6). Late trypsin mRNA levels at different times after feeding, in both methoprene-treated females and controls, were not significantly different (results not shown).

Discussion

The midgut epithelium of female *A. aegypti* is not fully developed at the time of adult emergence, and its cytodifferentiation, including the formation of microvilli, the rough endoplasmic reticulum (RER) and desmosomes, and the elaboration of the basal labyrinth, is completed during the following 2–3 days (Hecker *et al.*, 1971). A role for JH in the post-emergence maturation of *A. aegypti* midgut has been previously proposed based on the development of the RER (Rossignol *et al.*, 1982). The RER in the midgut is arrayed in prominent concentric formations termed 'whorls' (Bertram & Bird, 1961), and these arrays form within the first 3 days after adult emergence (Hecker *et al.*, 1971), disaggregate within 9 min after the mosquito ingests blood, and reform 2–5 days later. Removal of the corpora allata prevents the development of the RER after emergence, and prevents the redevelopment of the RER whorls following their disappearance after blood feeding. Treatment with a JH analogue restored these capabilities, suggesting that JH regulates RER development (Rossignol *et al.*, 1982).

Transcription of the early trypsin gene is part of the normal maturation of the midgut in the adult female (Noriega *et al.*, 1996b). Our results have shown that the developmental increase in the expression of the early trypsin gene is prevented by abdominal ligation, which isolates the midgut from the normal source of JH, the corpora allata. Low doses of a JH analogue or higher doses of JH III restored the expression of the early trypsin gene in ligated abdomens. Consistent with a role for JH in regulating early trypsin gene expression is the observation that methoprene application after feeding caused the amount of early trypsin mRNA in the midgut to reach, within 12 h, levels normally observed in controls at 40–60 h (Noriega *et al.*, 1996b). Besides accelerating the re-initiation of early trypsin

gene expression following a blood meal, topical application of methoprene immediately after emergence reduced the 10 h lag normally observed before the transcription of the early trypsin gene reached detectable levels.

To our knowledge, early trypsin is the first JH-regulated mosquito midgut gene to be described. Although we have shown that JH regulates the transcription of the early trypsin gene in a stage-dependent, dose-dependent and 'head-independent' manner at the level of transcription, we do not yet know whether JH directly affects early trypsin gene expression or whether the effect is mediated by some other factor produced in response to JH.

Experimental procedures

Insects

A. aegypti of the Rockefeller strain was reared at 27°C and 80% relative humidity under a 12 h light : 12 h dark photoperiod regime. Adults were supplied with a cotton-wool pad soaked in a 10% sucrose solution. Mosquitoes were fed a 100 mg/ml solution of pig γ-globulin (Sigma, St Louis, Mo.) as previously described (Noriega *et al*., 1994).

Abdominal ligations

Females were anaesthetized on ice. Abdominal ligations were performed by tying a fine thread at the base of the abdomen and removing the anterior portion as described by Hagedorn *et al*. (1977). The wound was sealed with tackiwax (Boekel Industries, Philadelphia, Pa.). Ligated abdomens were kept in a humidified chamber (Hagedorn *et al*., 1977). The juvenile hormone analogue (7*S*) Methoprene (Sandoz, Palo Alto) and juvenile hormone III (Sigma) were topically applied in 0.5 μl of acetone. Acetone alone was applied to control abdomens.

RNA isolation and characterization

Total RNA was isolated from females or abdomens using RNA binding glass powder as previously described (Noriega & Wells, 1993). RNA was separated by electrophoresis on 1.2% agarose gels under denaturing conditions using the formaldehyde method (Fourney *et al*., 1988). RNA was transferred to Nytran (Schleicher & Schuell, Keene, N.H.) and hybridized with an early trypsin cDNA (Noriega *et al*., 1996b), late trypsin cDNA (Barillas-Mury *et al*., 1991) or with a ribosomal DNA probe (Gale & Crampton, 1989), each of which were labelled using the Random Prime Labelling System (BRL) and [α-^{32}P]dATP (ICN, Irvine, Calif.). Hybridizations were performed under high-stringency conditions, as previously described (Noriega & Wells, 1993). The amount of radioactivity bound to individual samples on the filter was quantified using a Betascope (Betagene, Waltham, Mass.). Background was less than 1%.

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