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Regulation of hemolymph trehalose titers by insulin signaling in the legume pod borer, *Maruca vitrata* (Lepidoptera: Crambidae)

Md. Abdullah Al Baki[a], Jin Kyo Jung[b], Yonggyun Kim[a,*]

[a] Department of Plant Medicals, Andong National University, Andong 37629, Republic of Korea
[b] Division of Crop Cultivation and Environment Research, Department of Central Area Crop Science, National Institute of Crop Science, Rural Development Administration, Suwon 16429, Republic of Korea

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ABSTRACT

A disaccharide, trehalose, is a main hemolymph sugar of the legume pod borer, *Maruca vitrata* larvae, but its titers fluctuated with feeding activity. During diurnal feeding in the photophase, hemolymph trehalose remained at a relatively low level (69 mM) and increased (98 mM) during scotophase. Starvation significantly increased the hemolymph trehalose level, in which the elevation of trehalose titers was dependent on the non-feeding period. The down-regulation of the trehalose level during the active feeding period seemed to result from mediation of the insulin/IGF signal (IIS). Injection of a porcine insulin suppressed the trehalose level in a dose-dependent manner. Genes associated with IIS of *M. vitrata* were predicted from its larval transcriptome, and their expression was confirmed in different developmental stages and tissues. All seven IIS genes selected were expressed in all developmental stages and different tissues. Silencing of four IIS genes (insulin receptor, Forkhead box O, a serine-threonine protein kinase, target of rapamycin) by RNA interference significantly modulated the hemolymph trehalose level. Starvation treatment changed expression of two trehalose metabolism-associated genes (trehalose phosphate synthase (TPS) and trehalase (TRE)) as well as the IIS genes. Silencing of *TPS* or *TRE* expression significantly down- or up-regulated the hemolymph trehalose level, respectively. In addition, silencing of IIS genes altered both *TPS* and *TRE* expression, indicating a functional link between IIS and trehalose metabolism. These results suggest that nutrients obtained from feeding activate IIS of *M. vitrata*, which then down-regulates the hemolymph trehalose level by altering trehalose metabolism.

1. Introduction

Trehalose is a nonreducing disaccharide of two glucoses linked by an α [1→1] glycosidic bond and is known to be a major insect blood sugar [33]. In insects, relatively high levels (5 ~ 100 mM) of trehalose are stored in the hemolymph to provide a major carbohydrate nutrient to target cells [2]. Trehalose in the hemolymph facilitates absorption of digested glucose molecules from gut lumen to hemolymph because of a steep gradient in glucose concentration between two compartments [16]. In addition, trehalose acts as a stress protectant under starvation and low temperature [35].

Trehalose is synthesized from glucose by trehalose-6-phosphate synthase (TPS) containing two functionally distinct catalytic domains, in which the N-terminal domain catalyzes the production of trehalose-6 phosphate from glucose 6-phosphate and UDP-glucose, and the C-terminal domain then dephosphorylates it to generate trehalose [7]. In contrast, trehalase (TRE) plays a role in conversion of trehalose to glucose in various insect tissues [31]. As in mammals, the hemolymph sugar level in insects appears to be regulated by the action of two endocrine signals, insulin-like peptide (ILP) and glucagon-like peptide (adipokinetic hormone: AKH). Ablation of insulin-producing neurons in the adult pars intercerebralis increased trehalose level in *Drosophila* [3]. Conversely, insulin injection or overexpression of an ILP suppresses trehalosemia. Also, in a lepidopteran insect, *Spodoptera exigua*, ILP and AKH play crucial roles in maintaining a relatively constant hemolymph trehalose level [11,24].

The legume pod borer, *Maruca vitrata*, is a subtropical insect pest. Its larvae feed on leaves, flowers, and pods of leguminous crops [30,34]. However, recent climate change helps this species to colonize in a temperate region, including Korea. Crop damage caused by *M. vitrata* larvae in Korea was first found in an adzuki bean (*Vigna angularis*) field in 2004 [10]. Furthermore, *M. vitrata* can become cold-hardy through supercooling capacity and production of cryoprotectants [13]. Trehalose is a cryoprotectant of *M. vitrata,* because its biosynthesis appears to be up-regulated during rapid cold hardening [13]. Though it remains unclear how the hemolymph trehalose level is regulated in *M. vitrata*,

* Corresponding author at: Department of Bio-Sciences, Andong National University, Andong 36729, Republic of Korea.
E-mail address: hosanna@anu.ac.kr (Y. Kim).

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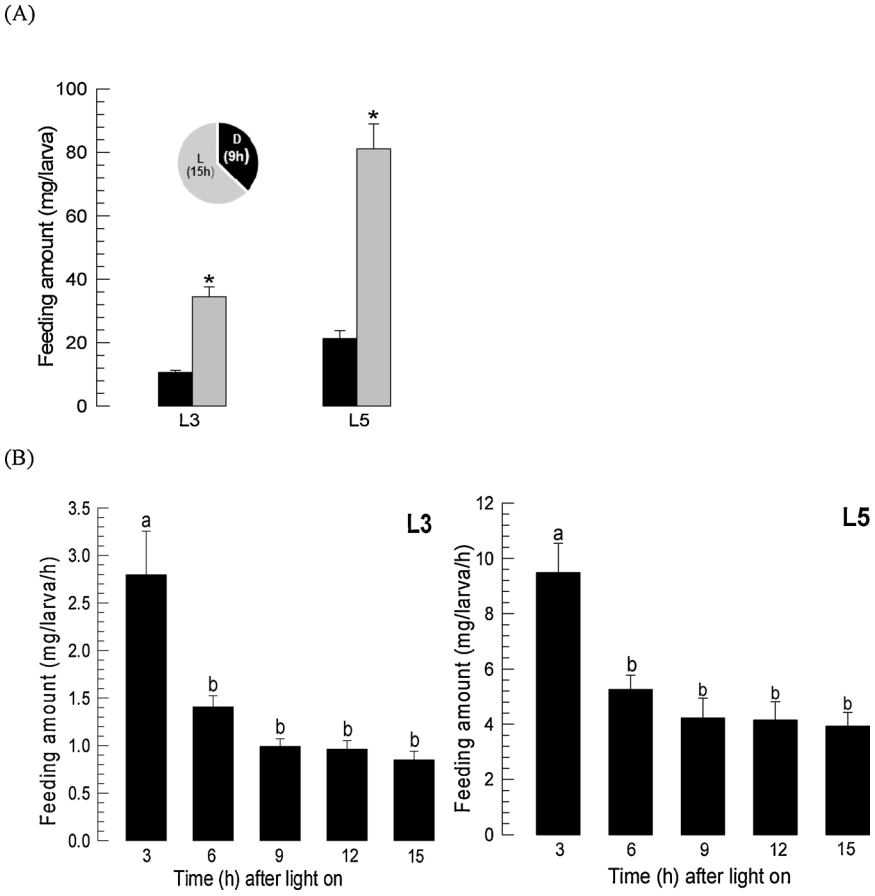

Fig. 1. Diel rhythm of feeding activity of *M. vitrata* larvae under 15:9 h (L:D) photoperiod and 25 °C temperature. Light-on was at 9 a.m. and light-off at 12 a.m. Newly molted (< 12 h), third ('L3'), and fifth ('L5') instar larvae were used for analysis. Experimental unit was a Petri dish (9 cm diameter) containing 5 larvae with artificial diet. Feeding amount was measured every 3 h by reduced diet weight per larva. Each treatment represented a 24-h measurement and was independently replicated three times. (A) Diurnal feeding activity. Asterisk above standard deviation bar indicates a significant mean difference between photophase and scotophase in each stage at Type I error = 0.05 (LSD test). (B) Variation of feeding activity during 15 h photophase since light-on at 9 a.m. ('3': 9 a.m. ~ 12 pm, '6': 12 pm ~ 3 pm, '9': 3 pm ~ 6 pm, '12': 6 pm ~ 9 pm, '15': 9 pm ~ 12 a.m.). Different letters above standard deviation bars indicate a significant difference between means at Type I error = 0.05 (LSD test).

AKH and ILP may play a role in regulating the trehalose level.

There is a host-associated variation in development of *M. vitrata* because of its polyphagous feeding behavior [1]. Subsequent study showed that the developmental difference may result from the post-transcriptional action of 13 micro RNAs against genes associated with cell signaling, metabolism, and metamorphosis [32]. However, the role of endocrine signals in the growth and development of *M. vitrata* are poorly understood. Nutrients are essential for all cell growth, but a systemic and coordinated growth of cells in multicellular organisms requires an endocrine signal, in which ILP or insulin-like growth factor (IGF) plays a crucial role in insects [21].

Since the first insect ILP was identified in the silkworm *Bombyx mori*, ILPs have been shown to regulate immature growth and adult reproduction in different insects [35,36]. Moreover, each insect species possesses multiple forms of ILPs; eight in *Drosophila melanogaster*, seven in the mosquito *Anopheles gambiae*, and 39 in *B. mori* [35]. Insect ILPs are homologous in molecular form to mammalian insulins as well as being functionally equivalent [17]. Especially, insect ILP/IGF signaling (IIS) shares high similarity with that of vertebrates [35]. An alteration in the IIS component genes led to severe growth retardation or overgrowth [20]. However, a functional correlation between IIS and hemolymph sugar level is relatively unclear in insect systems.

This study aimed to identify the IIS components of *M. vitrata* through its regulation of hemolymph trehalose level. IIS components of *M. vitrata* were predicted by interrogation of its larval transcriptome. After confirmation of their expression in *M. vitrata*, the functional association between IIS and the hemolymph trehalose level was assessed by RNA interference (RNAi) with each IIS component.

2. Materials and methods

2.1. Rearing of M. vitrata

A laboratory strain of *M. vitrata* was used in this study: It was collected from an adzuki bean field in Suwon, Korea, in 2004 and successively reared in a laboratory. Larvae of *M. vitrata* were reared on an artificial diet at 25 ± 1 °C, 16:8 h (L:D) photoperiod, and 60 ± 10% relative humidity. Larval developmental stages were classified according to their five instars (L1-L5). Under the rearing conditions, L3 and L5 lived for 3 days and 5 days, respectively. Adults were fed on 10% sucrose. For oviposition, a kitchen paper towel was provided in the adult cage.

2.2. Feeding rhythm assay

Newly molted (< 12 h) L3 and L5 larvae were used for assessment of feeding rhythm at different times in a day. Five larvae were kept in a 9-cm diameter petri dish with a piece (2 × 2 × 1 cm) of an artificial diet. Feeding amount was assessed every 3 h by measuring the reduction of the diet weight. Photoperiod (15:9 h, L:D) was maintained by light-on at 9 a.m. and light-off at 12 a.m. Temperature was constant at 25 ± 1 °C. Each 24-h run (an experimental unit) was independently replicated three times with fresh larvae that had not been used for a previous assessment.

2.3. Starvation treatment

Newly molted (< 12 h) L5 larvae were used. Three larvae were kept in an empty petri dish (9 cm diameter) at 25 ± 1 °C for different periods (0–24 h). All seven starvation regimes were applied to the larvae by setting four 6 h starvation regimes, two 12 h starvation regimes, and