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SHORT COMMUNICATION

The effect of BmNPV infection on protein metabolism in silkworm (*Bombyx mori*) larva

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Abstract

Grasseri is one of the most important diseases of silkworm with significant yield loss, which is caused by nuclear polyhedrosis viruses (NPV). In the present research the effect of this disease on changes of biochemical compounds which are related to protein metabolism in 5th instar larvae were studied. The larvae that showed the grasseri symptoms after contamination with 5.5×10^{-4} polyhedral/ml were assumed as infected treatment. The hemolymph of infected and uninfected larvae in 3 and 5 days after 4th molting were collected and its total protein, urea, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured. The results showed that the amount of all the compounds except urea were considerably different in both groups. Total protein had decreased in infected larvae but activity level of two aminotransferases significantly increased. Therefore, grasseri has a considerable effect on protein metabolism.

Key words: silkworm; nuclear polyhedrosis virus; protein metabolism; grasseri

Introduction

Silkworm is susceptible to many diseases caused by viruses and this is one of the main problems in sericulture every year. Generally, 70 % of damages caused by diseases are due to viruses (Anonymous, 1976). Among viruses, nuclear polyhedrosis viruses (NPV) in recent years have caused the highest damage to silkworm (*Bombyx mori*) in tropical regions (Sivaprasad *et al.*, 2003; Biabani *et al.*, 2005 a, b).

BmNPV affect midgut epithelial cells, trachea system, hemolymph cells, fat body, etc. The nuclear of middle and inner cells of silk gland are also sometimes invaded by this virus (Khurad *et al.*, 2004).

Baculovirus infection starts when inclusion body is taken in by the sensitive insects. Midgut fluid of lepidopteran larvae is totally alkali which digests the viral occlusion bodies. Consequently, virions are released into alimentary system and cross the peritrophic membrane. They combine with midgut epithelial cells and enter nuclei starting the first cycle of viral production and replication. These processes cause many biochemical changes in larvae which respond to these biological phenomena with changing many of its metabolisms to defend against pathogen invasion. Understanding and identifying these biochemical changes will be very important in discussing many biological stresses. Additionally, silkworm and BmNPV make a good model for studying the interaction between insect and virus. So, determination of the biochemical responses in silkworm against BmNPV could facilitate the control of agricultural pests (Gao *et al.,* 2006).

Biochemical compounds of silkworm have been investigated under many stress conditions as an appropriate marker. *Serratia marcescens* as one of the factors causing flasheri disease has been studied for the biochemical changes in silkworm hemolymph after infection and considerable decrease has been reported in total protein, carbohydrate and lipid (Sam Devdas *et al.*, 1994). Rami reddy *et al.* (1992) analyzed the effects of usi fly (*Exorista sorbillans*) parasitism on activity levels of aminotransferases and showed that stress increases the activity of the enzymes in silkworm. The researches have shown that infection with NPV does not have considerable effect on carbohydrates

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up to third day but with the time flow, the amount of carbohydrates is enhanced in infected larvae. However total lipid showed significant increase in the hemolymph from the first day of infection (Sarma *et al.*, 1994).

The effects of NPV on midgut enzymes of *Spodoptera litura* were investigated by Nathan *et al.* (2005) and it was demonstrated that alkaline phosphatase is decreased after infection by virus. Conversely, Matindoost (2006) showed that BmNPV had caused a considerable decrease in activity of this enzyme in silkworm after infection of a cell line established from silkworm embryo (Bm-EK1). Together with a drop in alkaline phosphatase activity, also many culture medium components such as cholesterol, urea and glucose showed considerable decrease. These findings confirm that viral infection has a significant effect on many cellular metabolisms.

Due to the importance of protein metabolism and its crucial involvement in many compensative and reductive processes involving biological stresses, in this study the effects of BmNPV infection on changes of some biochemical compounds which are related to protein metabolism was investigated.

Materials and Methods

BmNPV Inoculum preparation

Larvae infected with grasseri were collected from a field in north of Iran and their hemolymph was extracted into microtubes. The BmNPV in the larval hemolymph was confirmed by light microscopy of polyhedra. BmNPV polyhedra were purified as described by Sugimori *et al.* (1990). Samples were centrifuged at 10,000 rpm for 10 min to pelletize the BmNPV polyhedra. The polyhedra were then suspended in distilled water and quantified by hemocytometry as initial inoculums (Biabani *et al.*, 2005).

Silkworm rearing and hemolymph preparation

The eggs of a Japanese line, 103, were obtained from Iran Sericulture Research Centre (Rasht, Iran) and reared in the laboratory with standard rearing techniques under 25 ºC with RH of 75±5 % and photoperiod of 16L:8D (Krishnasawam, 1978). After 3rd molting, the larvae were divided into two groups including BmNPV infected larvae and uninfected ones. In each treatment 500 larvae were reared in two part of rearing room and both groups were fed by the leaves of Shinichenoise mulberry variety. A group of larvae were orally treated with 5.5×10^{-4} polyhedral/ml of BmNPV at the first day of 4th instar. Since disease symptoms were not observed in 4th instar in order to be certain that larvae have been infected, for each treatment 20 random samples were selected from 5th instar larvae in their 3rd and 5th days. Larval proleg was cut and hemolymph was collected in microtubes. One milligram phenylthiourea was added immediately to the tubes to prevent melanization. The samples were centrifuged at 14,000 rpm for 10 min. The supernatant was removed and kept in –20 ºC for analysis.

Biochemical analysis

The method of Lowry *et al.* (1951) was used for the total protein estimation. Hemolymph was diluted with distilled water and was added to alkaline copper reagent in microtubes. After 10 min 0.5 ml of Folin Ciocalteu's reagent was added to the mixture and microtubes were shaken thoroughly. The tubes were kept 20 min in room temperature for color development. The readings were taken on the spectrophotometer at 650 nm. For the reference, standard BSA was used. The concentration of urea was determined by measuring ammonia produced from urea, using a commercial urea assay kit (Chemenzyme Co., Iran). Alanine aminotransferase (ALT) (EC 2.6.1.2) and aspartate aminotransferase (AST) (EC 2.6.1.1) were measured utilizing Thomas (1998) procedure.

The t-test in SAS software was performed for determination of significant differences between the data in infected and healthy groups (SAS, 1997).

Results and Discussion

The results of this study have been demonstrated in Figs 1-4. As it is shown in Fig. 1, total protein considerable decreased in infected larvae compared to control. This reduction was observed in both sampling times in a way that total protein in the third day of 5th instar was 36.5 mg/ml in healthy larvae while in the same day the amount of protein in infected larvae was 7.5 mg/ml. This trend continued through fifth day of 5th instar and the amount of total protein in infected larvae decreased to 50 % of the control (Fig. 1).

It was previously reported that the total protein of hemolymph decreases in NPV infected silkworm larvae from the first to the seventh day of infection. This difference in the last larval day was more than 35 units decrease (Sarma *et al.*, 1994). Reduction of protein caused by NPV infection, which is called hypoproteinemia, not only occurs in silkworm but also it has been reported from many other lepidopteran species such as *Mamestra brassicae*, *Trichoplusia ni*, *Lymantria dispar*, etc (Young and Lovell, 1971; Pawar and Ramakrishnan, 1977; Mazzone, 1985).

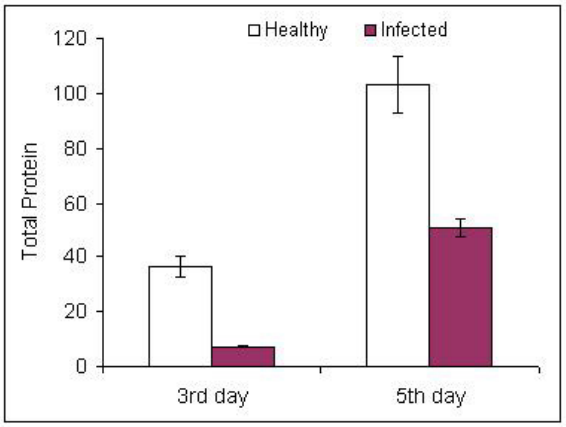

Fig. 1 The changes of total protein in infected and healthy groups of larvae (mg/ml)

Reduction of hemolymph protein could be due to the decrease of protein synthesis. It is assumed that virus activity is followed by the disruption of fat body cell and the lack of protein release to the hemolymph, hence, protein levels drop in hemolymph. In insects, the most important place for protein synthesis is fat body that is also the most sensitive tissue to NPV in silkworm. On the other hand, protein decrease could be the consequence of absorption process in midgut, because after entrance of virus to larval body, epithelial cells of midgut are invaded and therefore absorption process is interrupted. Although there are some researches that show there are not considerable changes in the quantity of some compounds in the insects infected by virus (Miao, 2002), often biochemical studies of silkworm larvae show that most of the stresses drop the amount of total protein in their hemolymph. It is assumed that silkworm compensates the deficiency of energy caused by the stress with intensive breakdown of proteins to amino acids and entering them to TCA cycle as a keto-acid (Nath *et al.*, 1997; Etebari and Matindoost, 2004a, b; Etebari *et al.*, 2006). It has been reported that high doses of baculovirus could increase the total protein in the cell culture of silkworm embryonic tissue. Matindoost (2006) suggested that the reason for this could be due to the bursting of cell membrane after cell death and release of cellular proteins, however, other factors have also been outlined.

Baculoviruses cause intensive interruption in hormonal regulation of larvae by having *egt* gene. This gene codes ecdysteroid UDP-glycosyl transferase and its expression inhibits in time ecdysis of larvae (Liu and Hou, 1985). This is while utilizing ecdyson and its derivatives affects protein metabolism in larvae infected by NPV. Liu and Hou (1985) demonstrated that compounds containing this hormone when treated on NPV infected larvae prevent intense changes in the amount and type of proteins within hemolymph.

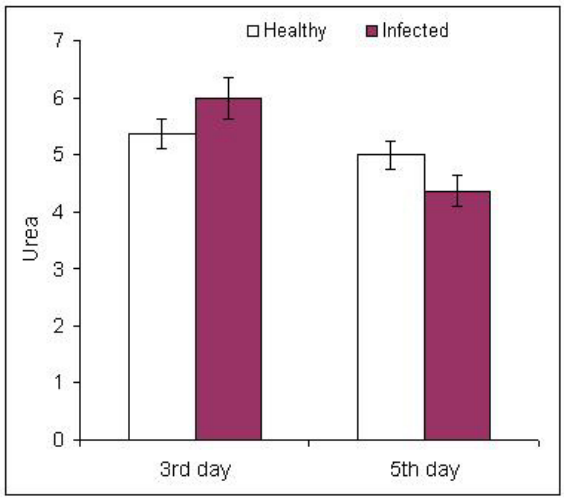

Fig. 2 The changes of urea in infected and healthy groups of larvae (mg/ml)

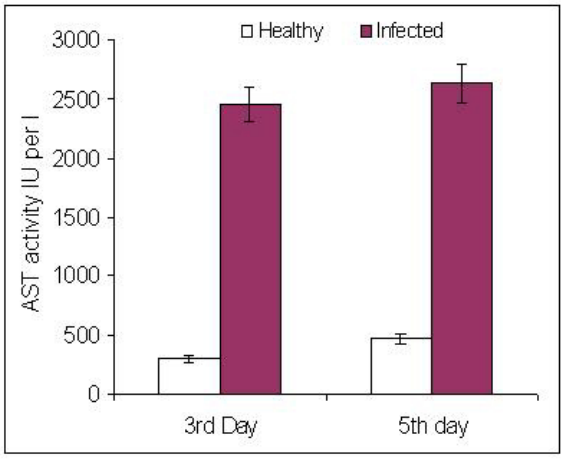

Fig. 3 The changes of AST activity in infected and healthy groups of larvae (IU/l)

Hemolymph urea did not have significant changes in two experiment times although it showed little difference (Fig. 2). Urea fluctuated between 4.37 to 6 mg/ml in uninfected and infected larvae. Urea as an excretory compound plays an important role in silkworm physiology and changes of its concentration in hemolymph of silkworm larvae are dependent to many factors such as larval stage, diet, etc (Sumida *et al.*, 1993). Urea changes are directly related to nitrogen metabolism and amino acids (Hirayama *et al.*, 1996). In this study not only no difference was observed between infected and uninfected larvae in the amount of urea but also no difference was evident in two sampling times.

Two amino transferases, AST and ALT, showed considerable increase in infected larvae (Figs 3, 4).

The mean activity of AST was more than five times higher than that in uninfected larvae. In detail, at third and fifth day of 5th instar the activity reached to 2,458.8 and 2,632.2 IU/l, respectively, while the activity in control at the same days was 294.7 and 474.5 IU/l. The changes of ALT follow the same pattern and its activity in infected larvae was more than triplicate.

It has been shown that silkworm larvae under different stress factors like parasitism by parasitoid flies, and/or after treatment with phosphorus pesticides and juvenile hormone analogues present fluctuations in the activities of amino transferase enzymes (Rami reddy *et al.*, 1992; Nath *et al.*, 1997; Singh *et al.*, 1997). Etebari *et al.* (2005) used the activity levels of these two enzymes as an appropriate biochemical marker to study the biodiversity of silkworm strains.

The transaminases are the important components of amino acid catabolism; which is mainly involved in transferring an amino group from one amino acid to another keto acid. The AST and ALT serve as a strategic link between the carbohydrate and protein metabolism and are known to be altered during various physiological and pathological conditions (Etebari *et al.,* 2005).

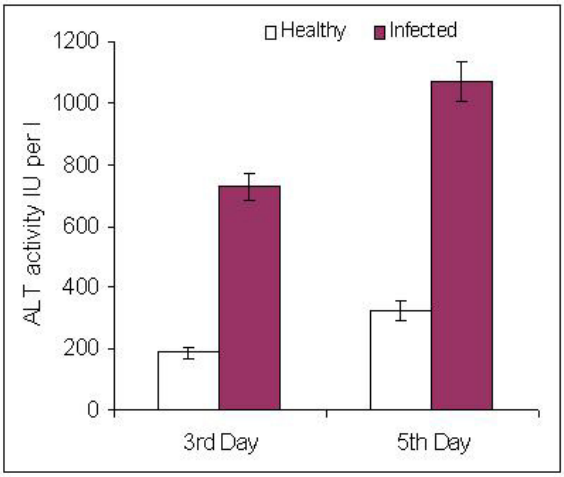

Fig. 4 The changes of ALT activity in infected and healthy groups of larvae (IU/l)

Generally ALT activity is mentioned as an index for breakdown of amino acids and AST as a sign for entrance of amino acid to glucogenesis process. Glucogenesis is a main path for sugar synthesis from non carbohydrate substrates (Lehninger, 1982). The carbon sources for glucogenesis in these series of reactions are amino acids and the activation of this metabolic pathway is usually associated by intensive decrease of free amino acids in fat body and hemolymph, because often with ALT activity, alanine transforms to pyruvate and enters the above pathway for energy supply. Although it has been reported that many factors such as larval strain, larval age, light period or rearing season and leaf type affect the changes of ALT activity levels (Khanikor *et al*., 1998; Devi and Sarma, 2000) viral infection is far more effective than other factors.

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