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Polyacrylamide Gel Electrophoresis of Proteins of the Meal-Worm, *Ephestia kühniella* Zeller

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Polyacrylamide Gel 전기영동법에 의한 밀얼룩명나방의 단백질 분리

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摘 要

貯藏穀類의 害虫인 밀얼룩명나방(*Ephestia kühniella* Zeller)의 變態에 따른 蛋白質 pattern을 調査하기 위하여 polyacrylamide gel 電氣泳動法으로 測定한 결과는 다음과 같다.

1) 變態에 따라 5齡幼虫에서 蛹期末까지 調査한 泳動相은 蛹前期에 12개, 終齡幼虫(♂)과 蛹後期(♀)에서 가장 많은 18개의 band가 나타났다.

2) Band의 數와 染色强度, 移動度를 비교하면 終齡幼虫까지 계속 增加하나, histolysis가 일어나는 蛹前期에서 減少하고 histogenesis가 일어나는 蛹後期에서 다시 增加하였다.

3) 性別에 따른 蛋白質 pattern의 變異는 일반적으로 암컷이 수컷보다 band가 많고 染色의 强度가 짙었다.

INTRODUCTION

The development and metamorphosis of insect are accompanied by striking changes in the protein pattern. The proteins contained in the haemolymph and tissues of a number of insect have been studied extensively by many workers (Whittaker, 1959; Hudson, 1965; Chen and Levenbook, 1966; Chippendale and Beck, 1966; Wang and Patton, 1968; Geest and Borgsteeds, 1969; Srivastave, 1970; Pasteur and Kastritsis, 1971; Pentz and Kling 1972). However, there are no other reports of the study on the change of general protein patterns of insect whole body during the metamorphosis of the insect, except for the works reported by several authors (Butler and Leone, 1966; Zamburlini and Danieli, 1970; Pasteur and Kastritsis, 1971). Butler and Leone (1966) studied that the protein pattern of *Tenebrio molitor*, beetle from the saline extracts of six selected stages in the life cycle. They found a total of 13 proteins by means of agar-diffusion, immunoelectro-

phoretic and turbimetric precipitin tests and disc electrophoresis. Zamburlini and Danieli(1970) investigated the *Drosophila hydei* proteins at different stages of larval development and identified 20 discrete bands at the end of the development. And Pasteur and Kastritsis(1971) studied the changes in protein patterns of whole-fly homogenates, haemolymph, fat body, and salivary glands, and found the existence of both qualitative and quantitative changes in them.

The present study was undertaken to determine the changes of patterns of soluble whole body proteins during growth and metamorphosis of meal-worm, *Ephestia kühniella.*

MATERIALS AND METHODS

Test insect

The insects used in this work were obtained from the Ephestia Stock Center (ESC) at the Zoological Institute, Köln University, West Germany and maintained on the corn-meal at the rearing box (approximately, 25°C±1). The experiments were carried out at the following developmental stages; the 5th instar larva, the 6th instar male and female larvae, male and female prepupae, 2-day-old male and female pupae and 8-day-old male and female pupae, and males can easily be distinguished from females by testis.

Preparation of homogenates

At the desired stage, the insects were collected and the wet weights were determined on an analytical balance. The homogenation was performed in a tissue grinders with a ground-glass pestle. The solvent used was 0.85% NaCl solution. The samples were thoroughly homogenized on ice and the homogenate was transferred to a Corex glass centrifuge tube. Centrifugation was carried out for 20 minutes at 3,000 rpm. The supernatant was carefully pipetted out with micropipette to avoid the lipid layer on top and used for the experiment.

Electrophoresis

Polyacrylamide gels for electrophoresis were prepared with Canalco's premixed solutions according to the technique of Ornstein and Davis (1964). The electrophoretic compartment was made at the Zoological Institute, Köln University and the power supply was obtained directly from Heiyinger Co., München, Germany. In this experiment, the writer used a 7.5% gel of polyacrylamide and Tris glycine buffer (pH.8.6), respectively. And 20μl supernatant was applied to each gel tube for obtaining an acceptable electropherogram, and a current of 3mA per tube was allowed during electrophoresis, which was stopped when the tracking dye (BPB) reached 1cm from the bottom of the tube. The electrophoresis run was

completed within 2 hours at room temperature. After the migration was complete, the gels were removed from the glass tube.

Stains

The gels removed were stained in a satulated solution of Amido Black 10B in 7% glacial acetic acid for 24 hours. Destaining was achieved in 24 hours by diffusing out in a 7% glacial acetic acid solution. The electrophoretic runs for each stage were repeated over 5 times.

RESULTS

Whole body protein patterns of the meal-worm, *Ephestia kühniella* at various developmental stages from the 5th instar larva to the late pupa were examined by means of acrylamide gel disc electrophoresis. The protein bands were numbered 1-18 according to their mobilities. A total of 18 protein patterns were detectable on the electropherograms of whole body homogenates.

As represented in Figs. 1 and 2, these eighteen bands were developed from the extracts of male of the 6th instar larva and female of the late pupa, respectively. Each developmental stage exhibited qualitative differences in their protein bands. In general, the protein bands were increased with the growth of larva, decreased at the prepupal stage, and at the early and the late pupal stages they were increased, again. The prepupal stage, particularly, is characterized by the absence of densely stained band 8. Among 18 bands some bands remained constant regardless of developmental stage (for instance, the bands, 3, 6, 8 and 15, were consistent throughout the stages), but the relative concentrations of their protein content varied greatly while some bands become visible in specific developmental stages. Bands, 1 and 2, which were detected at the larval stages, were not identified at the prepupel stage, but they were detected again at the early and the late pupal stages, and band 5 was identified throughout all the stages except the 5th instar larva. The strongly stained bands, 8 and 15 identified at the 6th instar male and female larval and the male and female pupal stages, were not detected entirely in the male at the prepupal stage but only band 14 was detectable in the female at the same period. In addition, some differences in electrophoretic patterns were found between male and female.

DISCUSSION

Changes in the protein pattern of the haemolymph during the development of the larva of insects show different functional conditions in the metabolism. The protein patterns of meal-worm *Ephestia kühniella*, were studied by Egelhaaf (1965, a,b) and Cölln (1969), using the technique of cellogel electrophoresis in the

past. Egelhaaf (1965 a,b) studied the inheritance of haemolymph proteins and separated 9 proteins, and Cölln (1969) later investigated the changes of ontogenetic pattern in the haemolymph of same insect and identified 10 protein fractions. However, the writer separated a total of 18 bands from the extracts of whole body of *Ephestia kühniella* by means of acrylamide gel electrophoresis which gives a high resolution of the protein fractions. The number of bands detected in this experiment is far higher than those reported by both Egelhaaf (1965a,b) and Cölln (1969). This difference might be due to the fact that both Egelhaaf (1965a,b) and Cölln(1969) used only the haemolymph for the experiment, while in the present study whole body including the haemolymph was used as a sample and, in addition, this difference might also be due to differences in the nutrition, or the technique methods. Cölln (1969) reported that the concentration of haemolymph protein of meal-worm was increased with the development of larva, especially, its concentration was in maximum at the last instar larval stage, and at the prepupal stage, it declined and again, it was increased at the pupal stage. And in addition, he mentioned that the numbers of protein patterns were distinctly reduced at the prepupal stage. These changes have also been observed in electropherograms of whole body samples. That is, the soluble proteins of whole body were increased from 13 bands in the 5th instar larva to a total of 14-18 in the last instar larva. These dropped to 12-13 bands in the prepupae, but 17-18 were detected in the late pupa. From Figs.1 and 2, marked qualitative differences in protein patterns were found between the sexes. Compared with males, the patterns of females appear characteristically, at various developmental stages. Band 14 was stained densely in the female of the 6th instar larva and band 15 in the prepupa. And bands, 1 and 2 appeared in the early pupa and bands, 4 and 7 in the late pupa, respectively. Geest and Borgsteele (1969) reported that no difference was shown in protein patterns of male and female larvae and pupae. The presence or absence of this difference presumably be related to the species of insect. This, however, requires for further study. Particularly the absence of band 8 is quite peculiar at the prepupal stage. And bands, 1 and 2 were absent at the male and female prepupae and early male pupa.

This probably be due to concentration of these proteins below the detectable level rather than to their total absence (Geest, 1969). The increase and decrease of protein patterns seem to be correlated with the intense histolysis and histogenesis in insect.

SUMMARY

The soluble whole body proteins of meal-worm *Ephestia kühniella*, at various

developmental stages have been studied by means of acrylamide gel disc electrophoresis. A maximum of eighteen bands were observed in the male of the 6th instar larva and the female of the late pupa. In general, the protein bands increased with the growth of larva, decreased at the prepupal stages, and at the pupal stages they increased again. The possible significance of these observations has been discussed. In addition, comparisons have been made of the protein patterns between the males and females in the developmental stages.

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Explanation of Figures

Fig. 1. Photograph of whole body protein patterns in *Ephestia kühniella* Zeller.

A: 5th instar larva
B: 6th instar male larva
C: 6th instar female larva
D: male prepupa
E: female prepupa
F: 2 day old male pupa
G: 2 day old female pupa
H: 8 day old male pupa
I: 8 day old female pupa

Fig. 2. Diagrammatic representation of whole body protein patterns in *Ephestia kühniella* Zeller.

A: 5th instar larva
B: 6th instar male larva
C: 6th instar female larva
D: male prepupa
E: female prepupa
F: 2 day old male pupa
G: 2 day old female pupa
H: 8 day old male pupa
I: 8 day old female pupa

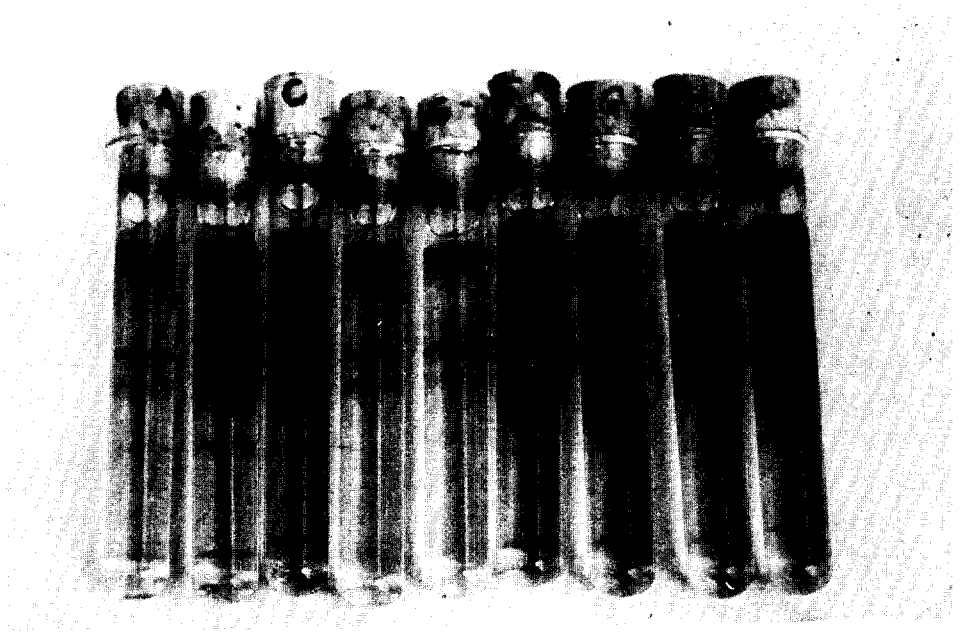

Fig.2.