Esterase and Leucine Aminopeptidase Zymograms in the Indian Meal Moth *Plodia interpunctella* During Development

Multiple molecular forms of different enzyme systems have been studied in many organisms, including insects. A number of electrophoretic studies of soluble proteins of insects have been made in recent years for the identification of the protein origin of the changes during insect development. The group of non-specific esterases is a favourable system, because of its presence in almost every living organism and its great heterogenity[1–10]. Different enzyme systems[11–17], including leucine aminopeptidase (LAP)[18–20], have been tried in insects by different electrophoretic techniques, because of their ontogenetic dependence.

So far, no data have been presented on the ontogenesis of esterases and LAP during the life cycle of *Plodia interpunctella*. The purpose of this investigation is to describe the electrophoretic pattern of the above enzyme systems in all developmental stages in the insect mentioned.

Material and methods. In the present investigation individuals of *Plodia interpunctella* (Hüb) (Lepidoptera: Phycitidae) of different developmental stages were used from a stock of our laboratory. A mixture of chicken mash, glycerine and honey in a 6:1:1 proportion was used as rearing medium. Temperature and humidity were kept constant at 28–30 °C and 40–50% respectively. Under these conditions, the stages of egg, larva and pupa were completed in 3, 17 and 7 days respectively. 48-hour-old eggs were used; 1, 8, 12 and 16-day-old larvae; 1, 4, and 7-day-old pupae and newly emerged adults. Individuals were frozen-thawed and squashed with a glass rod on an iced mortar with a drop of distilled water, in which sand was added for better homogenization. More than one individual were homogenized for samples of eggs and larvae up to the 12-day-old. For all other ages, the sample contained a single individual. Horizontal starch gel electrophoresis with a discontinuous buffer system was carried out according to the method described by ASHTON and BRADEN[21] as modified by CHRISTODOULOU[22]. Gels were prepared with 13% hydrolyzed starch (Connaught Medical Laboratories). The electrophoresis was performed at a constant voltage 42 V/cm for $2^1/_2$ h in a refrigerator at 4 °C. The zones of esterase activity were demonstrated using α-naphthyl butirate as substrate and Red TR salt as a coupling agent[2,23]. The zones of LAP activity were identified using L-leucyl-β-naphthylamide HCl as substrate and Black 'K' as coupling agent[18].

Results and discussion. The zymogram resulting from separation of soluble esterases of the insect examined is shown in Figure 1. The number of esterase areas according to their increasing electrophoretic mobility were 4 (IV, III, II, I). The zones of esterase activity had different times of appearance. Thus, 2 'strong' zones of area I appeared in 1-day-old larvae and were present up to the adults, while the 'faint' zone with the fastest mobility was present from 12-day-old larvae to 4-day-old pupae. Areas II and III were present in all developmental stages, while area IV appeared from 8-day-old larvae up to the adults. The maximum of relative esterase activity (amount of dye precipitation) for the majority of the zones, seemed to appear in the stage of the late larva. The relative activity of esterase-zones in both the earlier and later ages decreased significantly with minimum in the eggs and adults. Our observations concerning the esterase-activity in late larvae are different from those in some *Drosophila* species[2] where the maximum of activity was observed in adults. EGUCHI and SUGIMOTO[3], working on another Lepidopteron (*Bombyx mori*), found that the high intensity of esterase zones in the intestine appeared in the last larval stage. Our experimental results concerning the high esterase-activity in the late larval stage may be due probably to the intestine's behaviour.

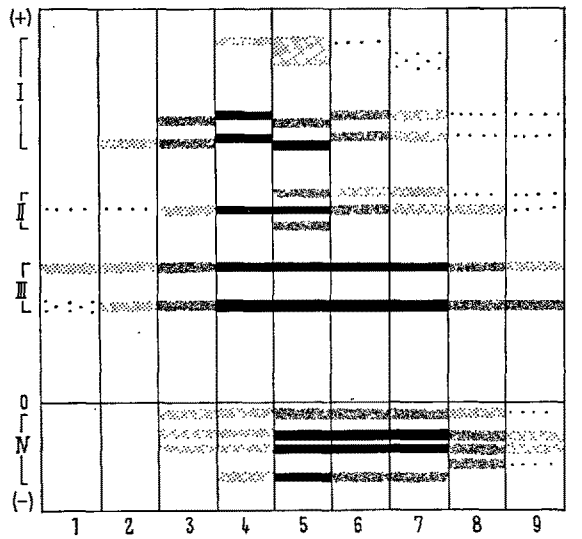

Fig. 1. Schematic picture showing the electrophoretic pattern of esterases during ontogenesis of *Plodia interpunctella*. 1. Eggs; 2–5. Larvae (1-, 8-, 12- and 16-day-old respectively); 6–8. Pupae (1-, 4- and 7-day-old respectively); 9. Adults.

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With regard to the LAP, 3 areas of activity appeared (Figure 2), characterized as areas III, II, and I according to their increasing electrophoretic mobility. Area I consisted of 1 to 3 zones. This area was present in all developmental stages with a maximum of relative activity in the pupal stage. Area II consisted of one zone of stable mobility present in all stages, with a peak of activity in 1- and 4-day-old pupae. Finally, area III consisted of 4 zones having different times of appearance. Thus, zone III_1, with the faster mobility, was present from 4-day-old pupae and thereafter zones III_2 and III_3 appeared from 12-day-old larvae up to the adults, while III_4 was observed from 8-day-old larvae and thereafter. The maxima of relative LAP-activity were observed in 4- and 7-day-old pupae. In some of the samples examined of 1-day-old pupae a faint zone of an intermediate mobility of areas I and II was visible. Generally, the highest LAP-activity was present in the pupal stage, particularly in mid and late pupae. The electrophoretic pattern of LAP in *Plodia* is in agreement with the findings of SAKAI et al.[18] concerning the stage where the highest LAP-activity appeared. The above observation supports the suggestion of SAKAI et al.[18] about the participation of exopeptidases, controlled by LAP-D locus, in the massive histolysis of larval tissues in pupal stage.

The absence of some zones in esterase and LAP zymograms of eggs, early and intermediate larvae may not indicate total absence of these molecular forms, but could be due to the sensitivity of electrophoretic technique[24]. On the other hand, the constancy of the majority of the molecular forms of esterase and LAP activity during metamorphosis in *Plodia*, supports the suggestion of PANTELOURIS et al.[2] that the genes responsible for the synthesis of esterases must be 'on' before and during metamorphosis.

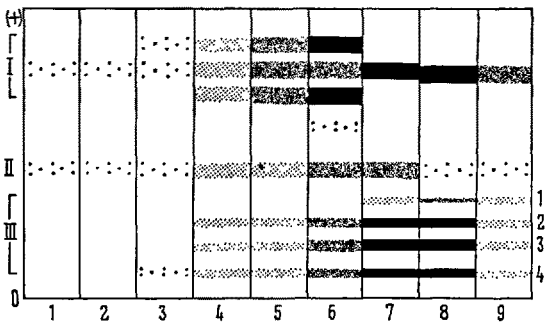

Fig. 2. Schematic picture showing the electrophoretic pattern of leucine aminopeptidase during ontogenesis of *Plodia interpunctella*. 1. Eggs; 2–5. Larvae (1-, 8-, 12- and 16-day-old respectively); 6–8. Pupae (1-, 4- and 7-day-old respectively); 9. Adults.

Zusammenfassung. Es wurden elektrophoretische Esterase- und Leucinaminopeptidase-Muster im Verlaufe der Entwicklung von *Plodia interpunctella* dargestellt.

H. A. PATARYAS[25], A. A. HARITOS and H. GELTI-DOUKA

Department of General Biology, University of Athens, Solonos 57, Athens (Greece), 2 September 1970.

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[25] Present address: Department of Medicine, University of Washington, Division of Medical Genetics, Seattle (Washington 98105, USA).

DNA and RNA Content in Diploid and Tetraploid Amphibians

The first cytological demonstration of polyploidy in vertebrates was reported by BEÇAK et al.[1] in the anuran *Odontophrynus americanus*. Several other examples of polyploidy were subsequently shown in other amphibians of the family Ceratophrydidae[2,3], Hylidae[4,5], and in fish[6,7].

The genus *Odontophrynus* includes several diploid species, as *O. cultripes*, *O. carvalhoi*, *O. occidentalis* and *O. americanus*, each with $2n = 22$ chromosomes[2,4,8]. The latter is confined to just a few localities. Another population of *O. americanus*, whose specimens are very similar to the diploid ones, was found to be tetraploid, exhibiting $4n = 44$ chromosomes[1,2]. The $4n$ populations have a wide geographical distribution, including several South American countries.

The existence of 2 populations phylogenetically very closely related, showing however different degrees of ploidy, constitutes an ideal system for a comparative study on gene expression. This peculiar situation gives rise to a few interesting questions. What happens to the mechanisms of regulation and transcription after the species duplicates all its genetical material? Is the production of RNA, proportional to the level of ploidy? Does the amount of protein of the tetraploid correspond to the DNA increase?

Starch gel electrophoresis of the albumin-like protein of the $4n$ *O. americanus* revealed polymorphism at this locus. 5 distinct phenotypes were found, showing that all 4 homologue genes are active in each animal but not necessarily synchronical[9].

RNA content. RNA measurements were made by spectrophotometric determinations according to the modified method of SCHMIDT and TANNHAUSER[10,11]. The animals were dissected and the kidneys immediately frozen. The tissue was homogenized in distilled water and the homogenate precipitated in $0.6N$ perchloric acid, centrifuged at $10{,}000 \times g$ for 10 min at 4 °C and washed in $0.2N$ perchloric acid. The precipitate was

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