ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. XLII, 2 SECTIO DD 1987

Instytut Chorób Zakaźnych i Inwazyjnych Wydziału Weterynaryjnego AR w Lublinie

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Native Proteins in Larval and Adult Haemolymph of the Colorado Potato Beetle (*Leptinotarsa decemlineata* Say)

Białka natywne hemolimfy larw i osobników dorosłych stonki ziemniaczanej (*Leptinotarsa decemlineata* Say)

Нативные белки гемолимфы личинок и взрослых особей колорадского жука (*Leptinotarsa decemlineata* Say)

INTRODUCTION

The role of blood proteins in fundamental physiological and developmental events of insect life have not yet been examined systematically. For this reason, the undegraded protein complexes of insect haemolymph receive a considerable amount of attention in order to understand all the mechanisms of their action and the means by which their activity in the insect's body is controlled. Although many evidences are already available about the synthesis and composition of insect lysozyme (6, 7, 13), the origin and nature of vitellogenins (14) and haemagglutinins (3, 19) far less is known about other inducible protective blood molecules of the cecropin (1, 4, 17) and attacin type (5); physiological role and biological importance of other soluble blood proteins is still not well defined. Obviously, conclusive evidences on the role of blood plasma proteins in development and reproduction, and in insect defence mechanisms are possible when the patterns of haemolymph proteins of an insect is defined accurately.

In this paper are presented the typical patterns of native haemolymph proteins of two well-defined stages of the Colorado potato beetle, *Leptinotarsa decemlineata* Say; two week-old larvae and adults 5–7 days post-emergence.

MATERIALS AND METHODS

Insects. Colorado potato beetles, *Leptinotarsa decemlineata* Say (*Coleoptera: Chrysomelidae*), two week-old larvae and adults 5–7 days post-emergence, were field collected from potato crops in August 1986 and directly bled to obtain haemolymph.

Collection of haemolymph. Blood samples were collected by gently piercing the thoracal lateral part of the beetly body and sucking the emerging haemolymph into an automatic micropipette.

An aliquot of 10 µl of blood was mixed with 50 µl of 1.0 M sucrose solution with a trace of phenylthiourea (Sigma) to prevent melanization by phenyloxidase activity. The haemolymphs, 22 individual samples of larval blood and 22 samples from adults, were stored frozen until used.

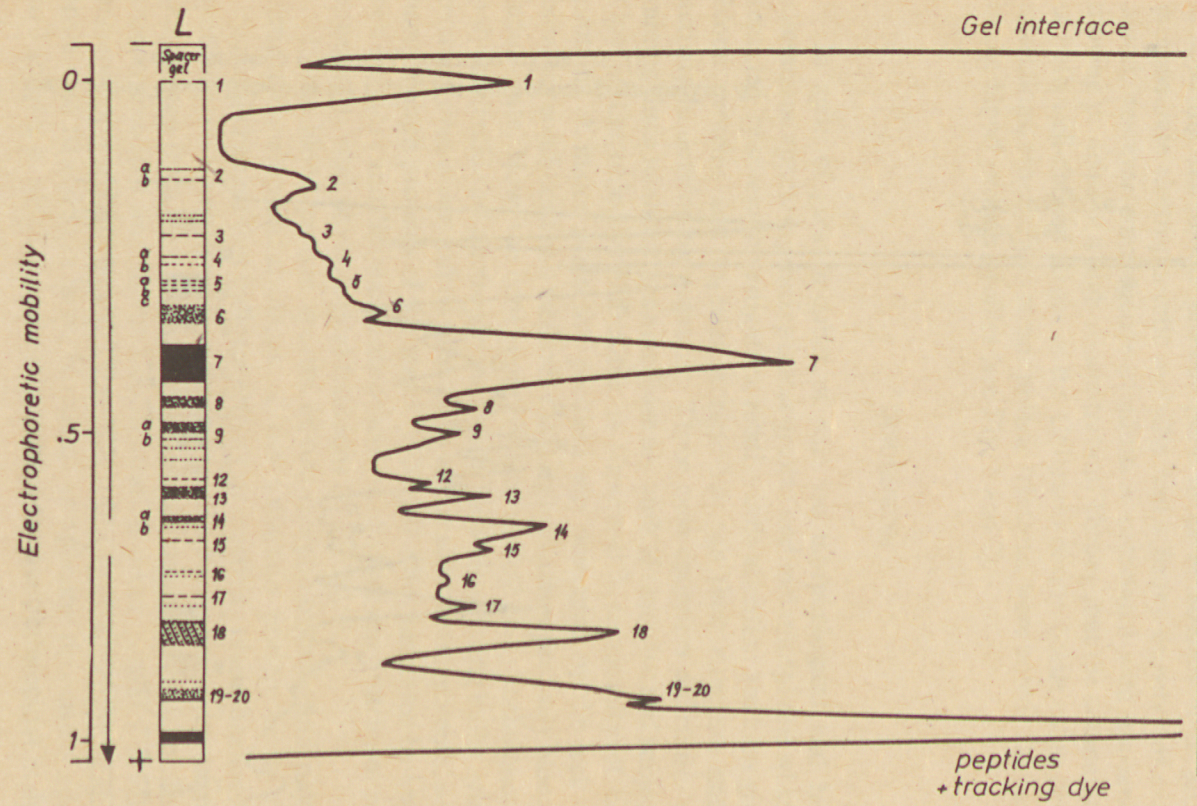
Polyacrylamide gel electrophoresis. To determine the distribution and developmental alterations of native protein fractions of normal blood of the beetle, the technique of Laemmli (9) under a non-denaturating buffer system (without sodium dodecyl sulphate added) was used. Twenty microlitre samples of haemolymph sucrose solution (equivalent to 3.3 µl of blood) were applied on the top of a 4.0% stacking gel. The separation was performed on a 10% acrylamide separation gel; length 9.0 cm, diameter 0.6 cm. Electrophoresis was carried out overnight at 18°C using a 0.1 M Tris-glycine buffer (pH 8.3) as a running buffer and a constant current of 1.5 mA/tube. After electrophoresis was termined, the gels were removed from the glass tubes and fixed for about 15 hr in the solution of 50% trichloracetic acid (TCA), stained for 24 hr in a 0.2% Coomassie brilliant blue R–250 in a solution of acetic acid-methanol-water (5:14:56, v/v) with 12.0% by weight of TCA, and then destained by several changes of acetic acid-methanol-water solution (4:24:56), at daily intervals. The staining procedure used enables to detect less than 1.0 µg of a single protein fraction.

Scanning of gels. The protein bands individualized by staining of the gels were scanned on a VT-Vitatron densitometer. The relative absorbance of each individual band was measured at a maximum absorbance of OD_{595} nm, and then the relative percentage of individual protein fraction migrating into a 10% acrylamide was calculated precisely.

RESULTS

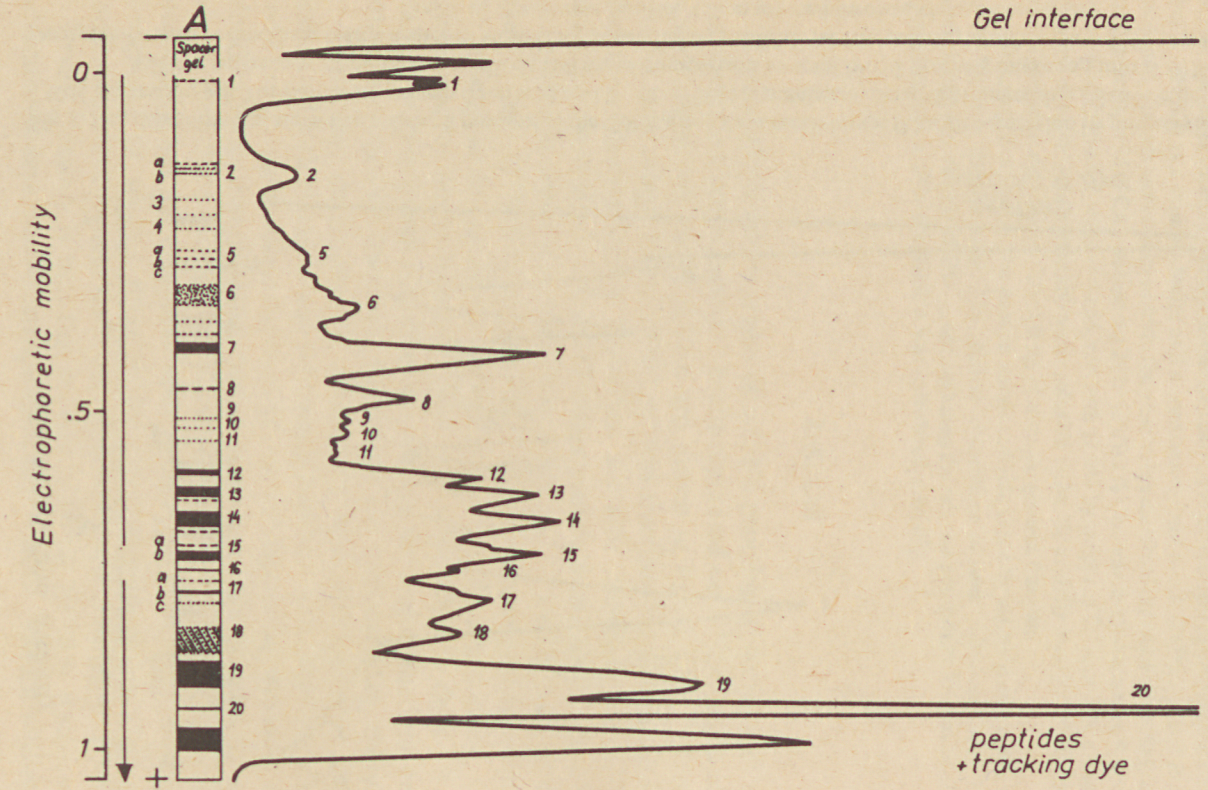
Typical electropherograms of soluble proteins of larval and adult haemolymph of the Colorado potato beetle separated on a 10% acrylamide gel under non-denaturating buffer system are shown in Fig. 1 and 2, respectively. The relative percentage of individual protein fractions visualized densitometrically and their electrophoretic mobilities are also presented in the figures. Disc electrophoresis yields at least 17 major protein fractions in larval blood (Fig. 1) numerated in order of increasing mobility and 18 well-defined protein bands in haemolymph of the adult beetle (Fig. 2). In addition, several minor proteins which are difficult to detect densitometrically because of a low content, are lettered at the respective major fractions. Now it is impossible to define if these faintly stained protein bands consist of subunits of the major fractions or if they form other heterogenous fractions of a similar electrophoretic mobility.

Generally, developmental alterations and distributional differences are associated with pupation and emmergence of the beetle. The lack of protein no 10 and 11 of an intermediate electrophoretic mobility in the protein spectra of larval blood indicates that these two components are specific proteins for adult stage. In adults, the minor proteins no 3 and 4 (together with subunits a and b) lower profoundly, but not disappear entirely, indicating that they themselves may be recognized as specific proteins for larval developmental stage. Again, the concentration of protein no 7 in adult blood is lower by about 2.5-fold than that in larvae. Possibly is that this protein of larval blood is composed of several subunits not separated electrophoretically. Almost 7-fold lowering the concen-



Fraction	Relative percent
1	1.79
2a,b	2.38
3	1.34
4a,b	1.94
5a,b,c	1.64
6	5.07
7	19.08
8	4.32
9a,b	6.41
12	4.32
13	4.17
14a,b	7.30
15	5.37
16	5.07
17	4.02
18	11.92
19–20	13.86

Fig. 1. Densitometric tracing of haemolymph proteins of the Colorado potato beetle larvae (L) after separation by polyacrylamide gel electrophoresis in a 10% separation gel. The particular protein fractions (no 1 — 19 — 20) are numbered along their increasing relative mobility. The figures denote the relative percentage of particular soluble proteins in comparison to total blood proteins migrating into a 10% acrylamide gel
Densytometryczne oznaczanie białek hemolizy stonki ziemniaczanej po rozdziale na żelu poliakrylamidowym w 10% roztworze. Poszczególne frakcje białek są oznaczone zgodnie z rosnącą ruchliwością elektroforetyczną



Fraction	Relative percent
1	2.34
2a,b	2.16
5a,b,c	3.29
6	5.54
7	8.39
8	4.33
9	1.21
10	1.82
11	0.87
12	4.15
13	6.23
14	8.30
15a,b	4.33
16	2.16
17a,b,c	8.65
18	6.92
19	17.21
20	12.11

Fig. 2. Adult blood. Details as for Fig. 1, but electropherogram marked A
Krew dorosłego osobnika (oznaczono jak na ryc. 1)

tration od protein 9 in adult apparently visualize developmental changes in the pattern of blood protein spectra. This protein fraction of larval blood is composed at least of two closely-spaced proteins lettered a and b. The concentration of proteins no 16 and 18 was about 200% higher in larval blood; however protein no 17 (together with minor fractions a, b and c) is about 2-fold more concentrated in adults. Other major soluble blood proteins have a comparable density in both developmental stages of the beetle (see Fig. 1 and 2). Comparing the blood protein patterns it is obvious that proteins of an intermediate electrophoretic mobility and a heavy density dominate in larval blood; however emmergence of adults is associated with appearance of fast-moving proteins of a high concentration. Throughout the comparative analyses of both well-defined stages of the Colorado potato beetle other developmental alterations, especially in minor protein fractions, were noted. They are presented in the blood protein profiles (see Fig. 1 and 2).

DISCUSSION

In spite of great number of data on morphological and histochemical alterations appearing during insect growth and metamorphosis which have been reported by several investigators, relative little attention has been given so far to blood proteins and to relationships between their structure and biological activity in developing organism. There exist countless difficulties when determining these relationships and those are both of objective and subjective nature. One of the objective difficulties is structural heterogeneity of haemolymph proteins. A major subjective problem was presented by the lack of standard bioassay conditions and methods enabling to examine precisely blood proteins in intact state. Now, the contemporary biochemical assay techniques, preferably polyacrylamide gel electrophoresis under non-dissociating buffer system enables to study even a very subtle differences and specific developmental alterations in haemolymph proteins of the insect (8, 20–23). They indicated by appearance of new fractions and disappearance others which represent proteins or polypeptides engaged in vital physiological functions (2, 11, 24).

In the blood protein spectra of two morphologically and physiologically distinct developmental stages of the Colorado potato beetle there occur structural changes that indicate activity or inactivity of mechanisms of selective synthesis or sequestering of proteins from the haemolymph by the fat body. As can be seen from Fig. 1 and 2, considerable variations both in the number of protein fractions and in their concentration occur between larval and adult haemolymph protein spectra. These alterations especially entire disappearance of protein no 10 and 11 in larval blood and an apparent decrease of adult proteins no 3 and 4 a, b, which are precisely synchronized with the course of development

may be assumed as typical for an individual developmental stage. The resulting changes reflect also that development is accompanied by qualitative and quantitative alterations of the electrophoretic patterns of the haemolymph protein pool.

Like in other insects, the blood proteins of the beetle may themselves be a subject of internal growth changes induced by hormones. Proteins are sequestered from the haemolymph by the fat body, this process appears to be selective, and the proteins stored are apparently utilized during adult differentiation. The synthesis and release of specific haemolymph proteins by the fat body of the growing larvae and the removal and storage of these proteins during later development are under hormonal control. Effects of juvenile hormones on synthesis of vittellogenic proteins were observed in adult females of Endopterygote insects, such as in *Leptinotarsa decemlineata* (10), *Manduca sexta* (18) or *Danaus* spp., (16). With respect to haemolymph proteins it appeared that some of the protein fractions may considerably change in quality and quantity after juvenile hormones application (12).

In addition, haemolymph proteins, especially lysozyme, attacin and cecropin type bactericidal inducible proteins are protectors of the normally functioning body because they together with cellular mechanisms, form the internal insect immune defence system (1, 15). An understanding of these complex responses mounted by the insect body as a defence against invading parasites or stress conditions is essential for the proper knowledge of the disease process. Therefore, the haemolymph protein spectra of normal beetle in respect to developmental variations (see Fig. 1 and 2) are valuable for further define of internal reactions of the insect host to microbial invaders or environmental stress conditions.

CONCLUSIONS

1. Notable changes in spectra of native blood proteins in larvae and adults of *Leptinotarsa decemlineata* are associated with pupation and emergence of the Colorado potato beetle.
2. Blood protein patterns of the beetle in respect to developmental variations are valuable for understanding effects of stress and microbial invasion on humoral defence mechanisms of the insect, used as a model for immunological studies.

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Wpł. 25 I 1988 r.

STRESZCZENIE

Rozdział elektroforetyczny hemolimfy larw w wieku 2 tygodni i osobników dorosłych w wieku 5–7 dni po przeobrażeniu stonki ziemniaczanej (*Leptinotarsa decemlineata* Say) wykazał istnienie wyraźnych zmian w składzie natywnych białek krwi związanych z rozwojem. Brak białka nr 10 i 11 o pośredniej ruchliwości elektroforetycznej w spektrum białek krwi larw wskazuje, że te dwa składniki białkowe hemolimfy są specyficzne dla osobników dorosłych. Wyraźne obniżenie poziomu białek nr 3 i 4 (łącznie z podjednostkami a i b) u osobników dorosłych pozwala na uznanie tych białek za swoiste dla stadium larwalnego. Stwierdzone różnice w obrazie białek rozpuszczalnych krwi wskazują na istnienie różnic w metabolizmie u dwóch odrębnych stadiów rozwojowych stonki ziemniaczanej.

РЕЗЮМЕ

Электрофоретическое разделение гемолимфы 2-недельных личинок и 5–7-дневных взрослых особей после видоизменения колорадского жука (*Leptinotarsa decemlineata* Say) показало, что существуют чёткие изменения в составе нативных белков крови связанных с развитием. Отсутствие белка № 10 и 11 со средней электрофоретической подвижностью в спектре белков крови личинок показывает, что эти два белковые компонента гемолимфы характерное для взрослых особей. Выразительное понижение уровня белка № 3 и 4 (включая подединицы а и б) у взрослых особей дает возможность считать эти белки как характерное для личиночной стадии явление. Обнаруженные разницы в картине белков растворимых крови свидетельствуют о выступлении разниц в метаболизме двух разных стадий развития колорадского жука.