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Newly Identified, Basic Hemolymph Proteins from Noctuid Species

Grace Jones*, Mietek Wozniak** , and Davy Jones

Department of Entomology, University of Kentucky,
Lexington, Ky 40546-0096

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A number of basic metamorphosis-associated proteins were identified from several noctuid species. All of these proteins have molecular weights in the range of 73,000 to 74,000. Two of the proteins in Trichoplusia ni and Heliothis virescens were found to be suppressible by a juvenile hormone analog.

There are a number of metamorphosis-associated proteins in insects that increase to high abundance just prior to pupation. Arylphorins, blue chromoproteins and lipoproteins have been the most intensively studied and the most well characterized of such proteins (1-3). They share many common characteristics in that they are lipophilic, contain carbohydrate, are acidic in nature and become increasingly prominent during the feeding stage of last larval stadium. Further, treatment of larvae with juvenile hormone (JH) or JH analogs does not seem to influence the abundance of these proteins during the final larval stadium (4).

This study reports the discovery of abundant, basic, metamorphosis-associated proteins in a number of noctuid species. The basic proteins are also shown to be suppressible by a JH analog.

MATERIALS and METHODS

Chemicals: Ampholines for establishing a pH gradient of 3.5-9.5 were obtained from LKB. Other chemicals for electrophoresis and staining were obtained from BioRad and Aldrich. Silver staining was performed as described (5), a method in which color indicates amino acid composition.

Electrophoretic analyses: Isoelectric focusing (pH 3.5-9.5) of hemolymph proteins was performed according to Jones et al. (6). Ten µl of hemolymph were applied to each lane of IEF gels. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the method of Laemmli and Favre (7). For two-dimensional analysis, proteins

*To whom correspondence should be addressed.

**On leave from Department of Biochemistry, Medical Academy, Wrocław, Poland.

Abbreviations used are: JH, juvenile hormone; JHA, juvenile hormone analog; IEF, isoelectric focusing; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

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were first subjected to pH 3.5-9.5 IEF (first dimension) and then to 7 % SDS-PAGE for the second dimension.

Insect rearing and hemolymph collection: Insects were reared according to Shorey and Hale (8). Hemolymph collected by piercing the prolegs of the larvae was centrifuged briefly at 5000 x g to remove cells and then stored at -70° C until use. Insect species used were: Trichoplusia ni (Hübner), Heliothis virescens (F.), Spodoptera exigua (Hübner) and Pseudoplusia includens (Walker) (all family Noctuidae).

Juvenile analog treatment: 100 nmoles of a juvenile hormone analog (JHA), fenoxycarb, were applied in 1 µl of ethanol to the dorsum of the larvae at the intramolt period before the final instar. Treated larvae were bled on day 2 (T. ni) or day 4 (H. virescens) of the last larval stadium. Control larvae were treated with ethanol.

RESULTS

IEF analysis of basic proteins in noctuid species: Basic proteins with pI 8.0-8.5 were found in all four noctuid species examined (Fig. 1a). Several of the species showed more than one protein band in this basic region. In these species in which more than one band was present, both

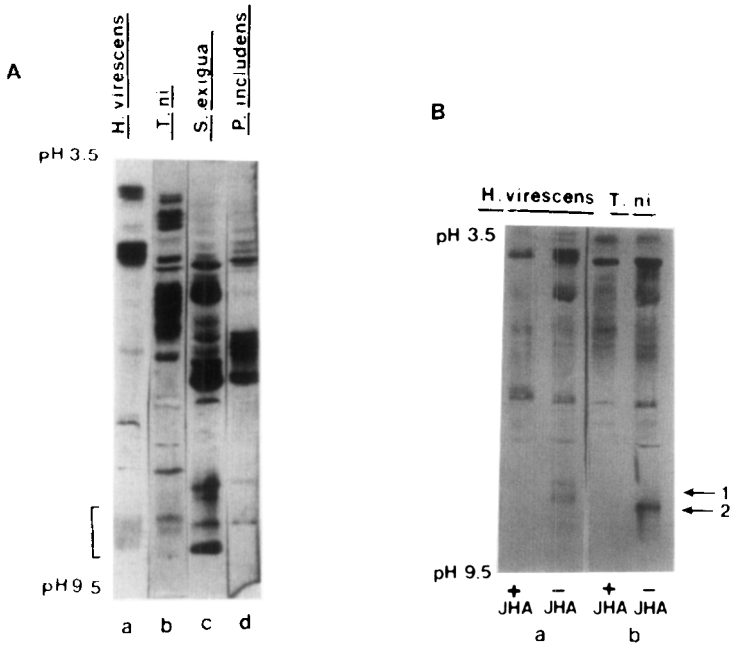

Fig. 1. A) Isoelectric focusing (pH 3.5-9.5) of hemolymph proteins from the late feeding stage of the last larval stadium of four noctuid species. a) Heliothis virescens, L5D4, 7 hr ALO, b) Trichoplusia ni, L5D2, 3 hr ALO, c) Spodoptera exigua, L5D3, 7 hr ALO, d) Pseudoplusia includens, L5D2, 3 hr ALO. Ten µl of hemolymph diluted 1:1 with dH0H were loaded per lane. Bracket marks the region of basic proteins. ALO - after lights on. B) Suppression of basic proteins of a) Heliothis virescens and b) Trichoplusia ni by the juvenile hormone analog fenoxycarb. Larvae were treated at or shortly after ecdysis to a final larval instar and bled at the time of natural occurrence of several abundant hemolymph proteins. Ten µl of hemolymph were subjected to IEF (pH 3.5-9.5), and then visualized with Coomassie blue. The results show several proteins which are suppressed by the JH analog and a number of proteins which are not. Arrows indicate two basic proteins of interest which are suppressible by the JH analog. +JHA - juvenile hormmone analog treatment; -JHA - control. The time points at which the insect were bled were the same as those in Fig. 3.

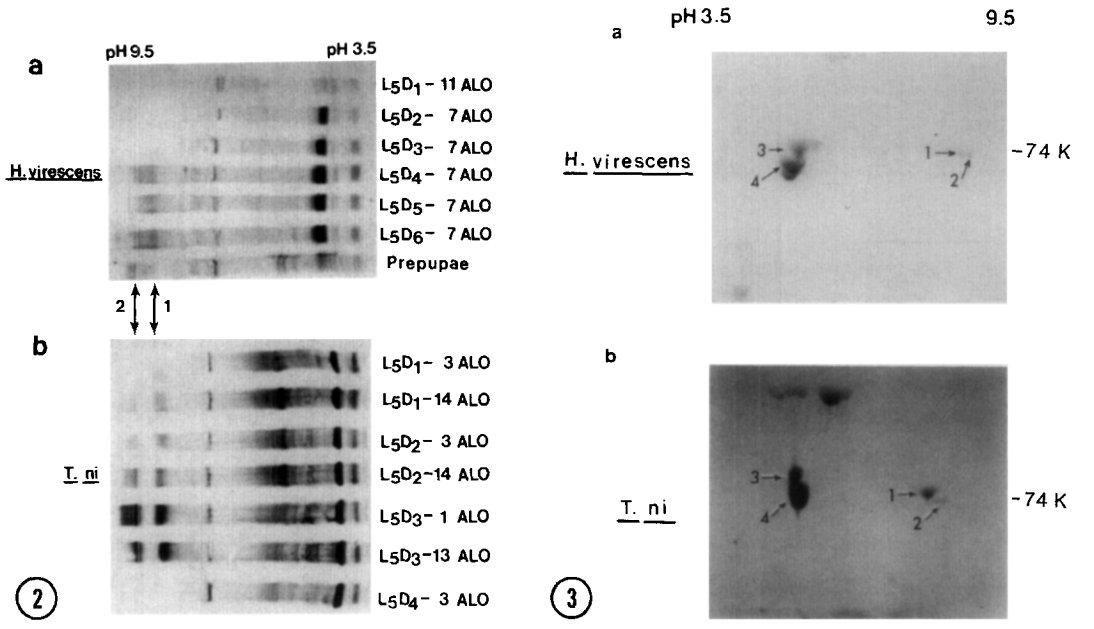

Fig. 2. Isoelectric focusing (pH 3.5-9.5) of hemolymph proteins showing the protein profile during development in the final larval stadium of a) Heliothis virescens and b) Trichoplusia ni. Each lane received 10 µl of hemolymph, diluted 1:1 with dHOH. Arrows indicate two basic proteins which appear during this stage of development and then decrease just prior to pupation. ALO-after lights on.

Fig. 3. Two dimensional electrophoresis (IEF, SDS-PAGE) of proteins in the hemolymph of late feeding stage, final stadium a) Heliothis virescens, L5D4, 7 hr ALO and b) Trichoplusia ni, L5D2, 3 hr ALO. Arrows 1 and 2 indicate two basic proteins in both insects with molecular weights near 73-74 kDa. Arrows 3 and 4 indicate groups of marker acidic proteins of 73-74 kDa whose sizes were determined previously (12). Proteins 1 and 2 correspond to the basic proteins shown in Fig. 2.

grey staining and orange staining basic proteins were observed following silver staining (not shown).

IEF analysis of developmental protein profiles of H. virescens and T. ni: In H. virescens, two basic hemolymph proteins began to appear on day 4 of the final larval stadium, reached the highest abundance on day 6 and disappeared just prior to pupation (Fig. 2a). In T. ni, two basic proteins appeared on late day 1, reached their highest level on day 3 and disappeared from the hemolymph during the prepupal stage (Fig. 2b). These two proteins were selected for further analysis.

Two-dimensional analysis of basic proteins: The two basic proteins (pI 8.3, 8.5) of both species were determined to be in a similar molecular weight range (Fig. 3). The two in T. ni, although similar in size, could still be distinguished on the basis of molecular weight, with the more basic one being slightly smaller. However, the two in H. virescens were not distinguishable by size separation in the second (SDS-PAGE) dimension.

IEF analysis of basic proteins after JHA treatment: The JHA treatment greatly suppressed the appearance of the basic proteins (Fig. 1b). A number of the other proteins remained unaltered, showing that the suppressive effect is specific for only certain proteins and is thus not a non-specific suppression of all proteins in general.

DISCUSSION

The present study has identified basic proteins (pI 8.0-8.5) in a number of noctuid species. These basic proteins are absent at the beginning of the final stadium, but increase to high abundance late during the final feeding stage. At present, the relationship of the basic proteins within and between species remains to be determined. However, data from T. ni (unpublished) showed that at least the two basic proteins for that particular species examined here are immunologically distinct. Therefore, at least some of the basic proteins in that species are more than just electrophoretic variants, i.e. they could be unrelated proteins with slightly different basic pIs.

A number of hemolymph storage proteins from different species of Diptera, Hymenoptera and Lepidoptera have been well studied (9-11). So far, all of the most well-studied ones have been acidic in nature (pI 6.0). The basic proteins described here were found to be suppressed by JHA. In contrast, most of the well studied acidic proteins are not suppressible by JHA. An exception is the recent description of an acidic, JH-suppressible protein in T. ni (12,13). It may be that in noctuids, in contrast to many other insect taxa, expression of certain abundant, metamorphosis-associated proteins is sensitive to JH or JHA treatment. The role of endogenous JH in regulating the basic proteins described here is as yet unclear. However, the results presented here suggest that the noctuid basic proteins should receive additional attention as possible models for the action of JH to regulate metamorphosis-associated proteins.

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