A CARRIER PROTEIN FOR JUVENILE HORMONE IN THE HAEMOLYMPH OF THE INDIAN MEAL MOTH: INTERACTIONS WITH EPIDERMAL TARGET TISSUE IN VITRO

S. M. FERKOVICH, D. L. SILHACEK, AND R. R. RUTTER
Insect Attractants, Behavior, and Basic Biology
Research Laboratory
USDA, ARS
Gainesville, Fla. 32604
U. S. A.

ABSTRACT: Evidence is presented for a carrier protein for juvenile hormone in the larval haemolymph of the Indian meal moth, *Plodia interpunctella*. The protein has an apparent molecular weight of 2.5×10^4 and selectively binds the hormone *in vitro*. Studies *in vitro* on the interaction of the carrier with target epidermis indicated that the protein protects the hormone from degradative enzymes and releases it at binding sites in membranes of the target cells.

INTRODUCTION: The importance of juvenile hormone (JH) in regulating the development of immature insects and the reproductive functions of adults is well known[1]. The hormone is secreted into the haemocoel by the corpus allatum and is transported by the haemolymph to target tissues. Because of the lipoidal nature of JH, it has long been assumed that the hormone must be transported in the haemolymph by a hydrophilic carrier. However, recently, the solubility of JH in aqueous solutions was shown to be in excess of the level needed to produce physiological effects[2]. Therefore, the more important function of a JH carrier is probably to protect the hormone during transport from interaction with non-target cells and degradative enzymes in the haemolymph and tissues [3,4].

Some evidence has been presented that proteins serve to transport JH in the haemolymph of several species of insects. The proposed JH carriers may be divided into 2 general groups of proteins. One group includes high molecular weight lipoproteins found to selectively bind the hormone or its analogs. Trautmann [5] first reported such *in vitro* binding of 2 JH analogs by 2 lipoproteins in the haemolymph of *Tenebrio molitor* larvae. Also, a high density lipoprotein with a molecular weight of 2×10^5 was reported to selectively bind *Hyalophora cecropia* JH in the haemolymph of pupal and adult saturniid moths[6]. Likewise, a JH binding lipoprotein (2.2×10^5 M.W.) was found in adults of *Locusta migratoria*[7]. The other group of suggested carrier proteins for JH includes proteins that are not lipoproteins and have a low molecular weight. For example, JH-binding proteins with molecular weights of 3.4×10^4 and 2.5×10^4 in the larval haemolymph of

Manduca sexta[2] and *Plodia interpunctella*[8], respectively, have been reported. In these insects, lipoproteins complexed with the hormone only when the JH-binding protein was saturated with JH. In addition, the JH carrier in *M. sexta* was shown to be necessary to protect the JH from enzymatic degradation in the haemolymph[9].

This paper is concerned with a JH binding protein that may serve as a carrier for JH in the larval haemolymph of the Indian meal moth, *P. interpunctella*. Our preliminary studies on the interaction of the protein with epidermal target tissue are presented.

EXPERIMENTAL: The collection of haemolymph, the labeling with radiolabeled JH, and the separation of the haemolymph proteins have been previously described[8]. Briefly, 0.1 ml of haemolymph was collected from larvae of three ages, early-, mid-, and late-fifth-instar (weighing 4, 8, and 20 mg/larva, respectively). Haemocytes were removed by centrifugation. The haemolymph was then labeled with *H. cecropia* JH (C_{18}) [7-ethyl-1,2-(^{3}H)], 14.1 Ci/mM (New England Nuclear Corp.), by incubation with hormone at 22°C for 15 min. Competitive binding studies were performed with an unlabeled mixture of isomers of *H. cecropia* JH (C_{18}) (Hoffmann-LaRoche, Inc.). The labeled haemolymph was then subjected to gel permeation with Sephadex G-200. The apparent molecular weight of the JH-binding protein was estimated from a calibration curve prepared with standard proteins on the same column. Specific labeling of the JH-binding protein in pooled fractions from the column and in whole haemolymph was studied by disc gel electrophoresis. JH was tentatively distinguished from its metabolites by thin layer chromatography.

Labeling of subcellular fractions from epidermis with ^{3}H-JH-carrier protein - The procedure for subcellular fractionation of epidermal tissue has previously been described[10,11]. Epidermal tissue from fifth-instar larvae (12 mg/larva) was washed several times with buffer. Care was taken to ensure that all adhering fat body material was removed. The tissue was homogenized, and ^{3}H-JH labeled carrier protein (obtained from the Sephadex column separation) was incubated with the homogenate at 4°C for 30 min. The homogenate was then centrifuged at 20,000 g to sediment the particulate material. The resultant pellet was washed once to remove unbound radioactivity and fractionated on a sucrose density gradient.

RESULTS AND DISCUSSION: Gel filtration of haemolymph from early fifth-instar larvae incubated with ^{3}H-JH showed that the radioactivity eluted between the 2 major absorption peaks (Fig. 1). The material associated with the label had an apparent M.W. of 2.5×10^4. Thin layer analysis of label indicated that 95% of the radioactivity was intact JH[8]. A similar 2.5×10^4 M.W. JH-binding material was also observed in the haemolymph taken from older larvae (mid- and late-fifth instar). Disc gel electrophoresis of the JH-binding material revealed that the JH carrier was a protein [8]. That the suspected JH-carrier protein was not a lipoprotein

was evident from its low molecular weight and the fact that it could not be stained with Sudan Black B and lipid crimson, two lipoprotein stains.

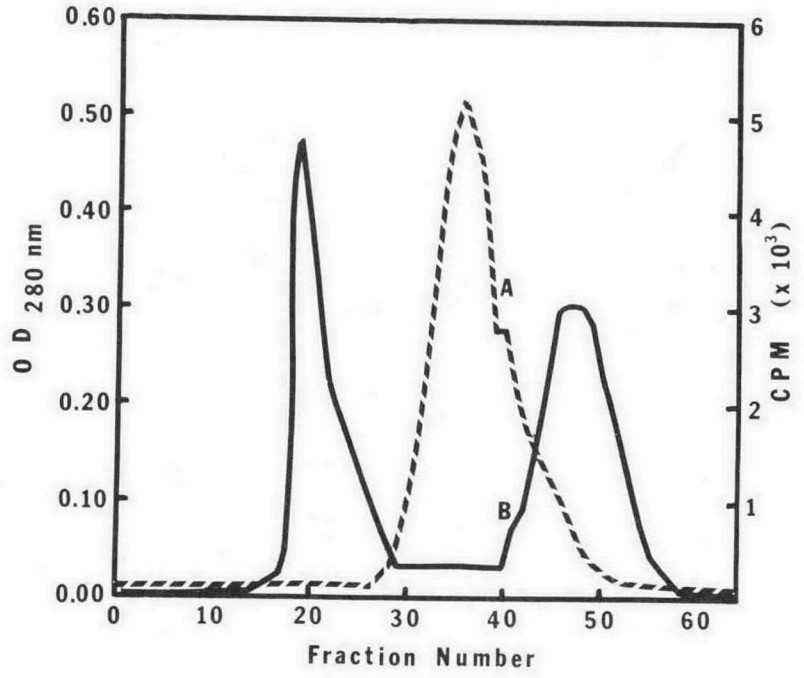

FIGURE 1. Elution pattern on Sephadex G-200 of 0.1 ml haemolymph incubated with ^{3}H-JH (2.5×10^{-8}M) for 15 min at 20°C. A, radioactivity; B, absorbance at 280 nm. For details see [8].

Lipoproteins with high molecular weight have been suggested as carriers of JH in saturniid moth pupae and adults[6] and in adult *L. migratoria*[7]. In this study, lipoproteins were present in the high molecular weight absorption peak (first peak eluted from the column, Fig. 1) but did not become labeled unless excessive concentrations of JH were used[8]. Preincubation of haemolymph from late-instar larvae with unlabeled JH before addition of the ^{3}H-JH eliminated the characteristic peak that resulted when only ^{3}H-JH was incubated with the haemolymph (Fig. 2). More important was the appearance of label coinciding with the lipoproteins in the high molecular weight absorption peak. These results indicated that the unlabeled JH saturated the binding sites on the 2.5×10^{4} M.W. JH-binding protein, which minimized subsequent binding and exchange. Presumably, the lipoproteins were either not saturated with unlabeled JH, or they exchanged JH more readily because of their nonspecificity in binding the JH molecule. Our results thus corroborated the observations of Kramer et al.[2], who reported a moderate molecular weight carrier in the haemolymph of *M. sexta*. Lipoproteins in this insect were also found to bind

JH only when the hormone was in excess.

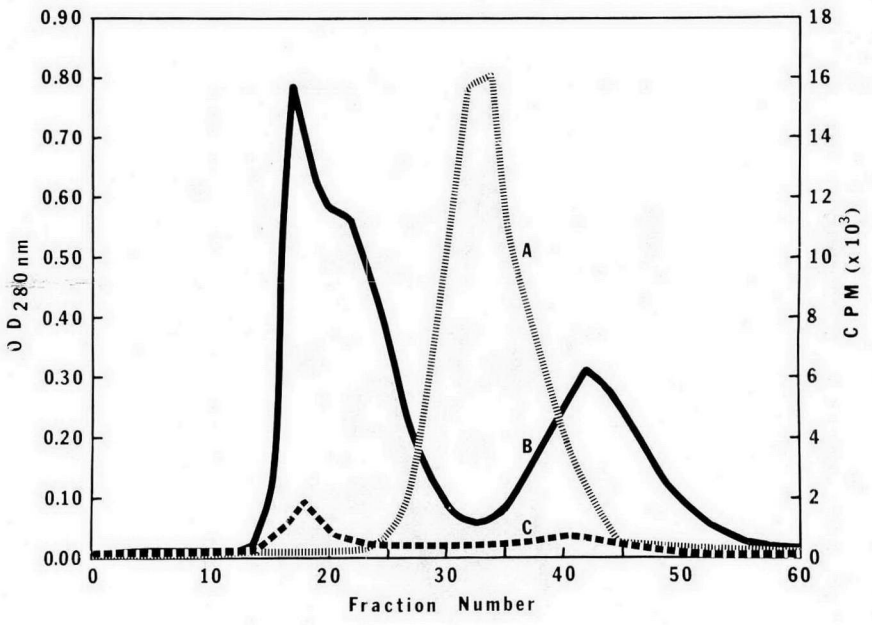

FIGURE 2. Elution pattern of 0.1 ml haemolymph on Sephadex G-200. A, haemolymph incubated with ^{3}H-JH (5×10^{-8}M) alone; B, absorbance at 280 nm; C, haemolymph preincubated with cold *Cecropia* JH (3.2×10^{-2}M) for 15 min at 20°C prior to incubation with ^{3}H-JH (5×10^{-8}M) for 15 min at 20°C. For details see [8].

In addition to transport of the hormone from the secretory glands to target tissue, a JH carrier could also protect the hormone from degradative enzymes in the haemolymph and tissues[9]. In the absence of the JH-binding protein, 87% of the hormone was rapidly degraded in an epidermal homogenate. However, in the presence of the JH carrier, only 13% of the hormone was degraded, indication that the carrier protects JH from enzymatic degradation.

A JH carrier would also be expected either to release the JH or bind as a JH complex at specific receptor sites in target tissue. Moreover, the JH carrier in *M. sexta* has been suggested as necessary for the recognition of the hormone at its receptor sites[9]. Table I indicates the resultant labeling of five particulate fractions from the epidermal homogenate incubated with the labeled JH binding protein. Binding was highest in the fraction at the 0-0.8 molar interface of the sucrose gradient that contains membrane-bound RNA and protein[11]. The chemical similarity of this fraction to the ribonucleoprotein receptor reported in pupal epidermis of *T. molitor*[12] suggests that the JH-protein

complex may have interacted with a similar hormone receptor in the epidermis of *P. interpunctella*.

TABLE 1. Distribution of radioactivity in particulate fractions obtained from a sucrose gradient separation of epidermal homogenate incubated with the radiolabeled JH binding protein[1].

Sucrose interface (m/liter)	Cpm/mg protein
0 - 0.8	70,300
0.8 - 1.0	13,213
1.0 - 1.2	15,921
1.2 - 1.5	3,405
1.5 - 1.8	4,730

[1]For details see [11].

If the observed labeling of epidermal fraction represents receptor-type binding, the JH-carrier complex may not be necessary for recognition of the hormone at its receptor sites because labeling of the particulate portion of epidermal tissue (free of soluble hydrolases) with ^{3}H-JH alone resulted in a similar binding pattern [11]. Thus, the main function of the JH-binding protein in *P. interpunctella* may be to protect the hormone from enzymatic degradation during transport to target cells.

The JH-binding protein is currently being purified and characterized. Studies are also underway to define the biochemical parameters involved in the mechanism of JH transport in *P. interpunctella*. From a practical standpoint, it may be feasible to develop an anti-JH compound that interferes with transport of endogenous JH to its target sites. Then by tying up the JH carrier with an anti-JH compound, endogenous JH would be left susceptible to enzymatic degradation. This, in effect, should lower the JH titer of the haemolymph in the immature forms of *P. interpunctella*. Such an alteration in the JH titer of the haemolymph would be expected to induce precocious molting and subsequently lead to the death of the insect.

REFERENCES:

[1] Schneidermann, H., "Insect hormones and insect control", p. 3, Insect Juvenile Hormones (Menn, J. J., Beroza, M., Eds.). Academic Press, New York (1972).

[2] Kramer, K. J., et al., The juvenile hormone binding protein in the haemolymph of *Manduca sexta* Johannson (Lepidoptera: Sphingidae), Proc. Natl. Acad. Sci. USA 71 (1974) 493.

[3] Slade, M., Zibitt, C. H., "Metabolism of *Cecropia* juvenile hormone in insects and in mammals", p. 155, Insect Juvenile Hormones (Menn, J. J., Beroza, M., Eds.), Academic Press, New York (1972).

[4] Whitmore, D., Whitmore, E., Gilbert, L. I., Juvenile hormone induction of esterases: a mechanism for the regulation of juvenile titer, Proc. Natl. Acad. Sci. USA 69 (1972) 1592.
[5] Trautmann, K. H., *In vitro* studium der tragerproteine von ^{3}H-markierten juvenilhormonwirksamen Verbindugen in der haemolymph von *Tenebrio molitor* L. Larven, Z. Naturforsch. 27b (1972) 263.
[6] Whitmore, E., Gilbert, L. I., Haemolymph lipoprotein transport of juvenile hormone, J. Insect Physiol. 18 (1972) 1153.
[7] Emmerich, H., Hartmann, R., A carrier lipoprotein for juvenile hormone in the haemolymph of *Locusta migratoria*, J. Insect Physiol. 19 (1973) 1163.
[8] Ferkovich, S. M., Silhacek, D. L., Rutter, R. R., Juvenile hormone binding proteins in the haemolymph of the Indian meal moth, Insect Biochem. (In press).
[9] Sandburg, L. L., Role of juvenile hormone esterases and carrier proteins in insect development, Nature (In press).
[10] Ferkovich, S. M., Rutter, R. R., Anthony, D. W., Spectrophotometric measurement of juvenile hormone binding in subcellular components of the Indian meal moth, J. Insect Physiol. 20 (1974) 1943.
[11] Ferkovich, S. M., et al., Juvenile hormone binding in epidermal target tissues of the Indian meal moth (In preparation).
[12] Schmialek, P., Ribonucleoprotein particle in epidermis cells as the receptor for juvenile hormone, Nature 245 (1973) 267.