J. steroid Biochem. Vol. 35, Nos 3/4, pp. 449–455, 1990
Printed in Great Britain. All rights reserved

0022-4731/90 $3.00 + 0.00
Copyright © 1990 Pergamon Press plc

A NEW METHOD OF DETECTING HORMONE-BINDING PROTEINS ELECTROBLOTTED ONTO GLASS FIBER FILTER: JUVENILE HORMONE-BINDING PROTEINS FROM GRASSHOPPER HEMOLYMPH

LINDA S. JEFFERIES and P. ELAINE ROBERTS*
Department of Entomology and the Cell and Molecular Biology Program, Colorado State University, Fort Collins, CO 80523, U.S.A.

(*Received* 17 *May* 1989; *received for publication* 11 *October* 1989)

Summary—We have developed a new method to identify juvenile hormone (JH)-binding proteins blotted onto glass fiber filter (GFF) after electrophoretic separation. Insect JH regulates reproduction in the two-striped grasshopper, *Melanoplus bivittatus*. A number of proteins are involved in the delivery of JH from its site of synthesis to the nuclei of fat body cells where it acts to induce vitellogenesis. To identify JH binding proteins, hemolymph was separated by PAGE, electroblotted onto GFF, and incubated in [10-^{3}H]JH-III. The amount of hormone bound by blotted proteins increased with the amount of protein on the filter, was competitively displaced by excess non-labeled hormone, and was affiliated with individual bands on fluorograms of proteins blotted after electrophoretic separation. GFF etched with trifluoroacetic acid was better than nitrocellulose, Zeta Probe, cellulose acetate or unetched GFF. Phosphate (pH 6.0–7.3) or Tris buffers (pH 7.3–8.0) worked equally well for the procedure. Unbound hormone was easily removed by short washes in buffer, and adequate binding for detection was achieved in a 15 min incubation. Preliminary data suggest that this technique may be used to detect receptors, carriers, and binding proteins of steroid hormones.

INTRODUCTION

Exposing electroblotted proteins to radioactive hormone provides a unique means to recognize binding proteins (BPs), carriers, and receptors through steps of purification. Polyacrylamide gel electrophoresis (PAGE) is an important tool for characterization of hormone binding proteins. However, these proteins are not easily identified after separation by PAGE. Since most hormones are not covalently bound to BPs, protein–hormone complexes may not be maintained during electrophoresis. With juvenile hormone (JH; methyl (2E,6E) 10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate; Fig. 1), several tactics have been used to identify JHBPs. [10-^{3}H]JH-III has been covalently linked to *Manduca sexta* and *Locusta migratoria* hemolymph BPs by irradiation with u.v.-light [1]. However, this method has not been successful with the two striped grasshopper, *Melanoplus bivittatus* [2]. The specific activity of [10-^{3}H]JH-III is so low and so few molecules remain bound after PAGE that only BPs with high capacity are detected. In fact, photoaffinity labeling with [10-^{3}H]JH-III was no better than when the hormone was not covalently linked. Synthetic photoaffinity analogs of JH such as EFDA (10,11-epoxy(2E,6E)farnesyl diazoacetate) [3–6], have also been used for covalent tagging of BPs. However EFDA tends to label many proteins, so the JH binders can only be detected by competitive displacement of [10-^{3}H]EFDA with unlabeled JH-III [2, 4]. There are other differences in detection of BPs that depend on the type of ligand used [4, 7–9]. In light of these results, we looked to electroblotting as a technique which would allow detection of JHBPs by binding JH to protein after separation by PAGE.

The detection of binding proteins blotted from a gel onto a solid medium has several advantages. First, the hormone is not expected to remain with the BP through PAGE, so covalent linkage of hormone and BP is unnecessary. Furthermore, by incubating blots in radiolabeled hormone, the amount of hormone needed for incubation can be minimized, and non-specific association of the hormone with the gel can be avoided. In addition, the technique would allow comparison of the proteins that bind JH with those that bind JH-analogs.

This paper describes an electroblotting technique which uses acid etched glass fiber filter (GFF) for the blotting medium. This medium can also be used for amino acid sequencing [10]. Blots incubated in [10-^{3}H]JH-III showed that proteins in two bands consistently bound JH. While juvenile hormone is a terpenoid, its action in gene regulation has much in common with the steroids. Although this procedure

*To whom correspondence should be addressed.

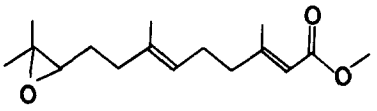

Fig. 1. Juvenile hormone III.

was developed for our insect system, it may be adapted for other hormones. Preliminary experiments indicate it has potential for detecting estrogen binding proteins.

EXPERIMENTAL

Sample preparation

Hemolymph was prepared from mature *M. bivittatus* females as described by Roberts and Jefferies [11]. Hemolymph phenyloxidase activity was inhibited by phenylthiourea. After 30–60 min at 0°C, the clotting proteins were removed by centrifugation at 100,000 *g* for 1 h. This hemolymph was used immediately or stored at −80°C until needed.

Dot blotting

A dot blot procedure for an immuno-binding assay by Shiebler and Greengard [12] was adapted for use with JH. Dot blots allowed evaluation of various blotting media and of protocols for hormone incubation and washing. Disks of blotting media were soaked in distilled H_2O for 5 min and dried with a stream of air. A total of 20 μl of sample was applied and dried onto the disks in three fractions. The disks were preincubated for 15 min in TN buffer (50 mM Tris, pH 7.3 and 200 mM NaCl) to rehydrate the filter and the sample before incubation. Disks were incubated 15 min in 10 nM [10-^{3}H]JH-III (New England Nuclear, Boston, Mass) in TN, washed four times for 5 min in approximately 50 ml TN, dried and counted by scintillation spectroscopy. The JH incubations described above were done in medium sized polystyrene weigh-boats because we found little JH adsorbed to these containers. Specific binding of JH to proteins was determined by dot blotting duplicate sample sets, and subtracting the amount of JH bound after incubation in [10-^{3}H]JH-III plus 100-fold excess unlabeled JH-III from values for samples incubated in [10-^{3}H]JH-III alone.

Selection of blotting medium

Nitrocellulose (BioRad, Richmond, Calif.), Zeta Probe (BioRad), cellulose polyacetate (Gelman, Ann Arbor, Mich.), and GFF (Gelman type A/E) were tested to determine which allowed blotted proteins to bind [10-^{3}H]JH-III quantitatively with the least background of adsorbed hormone. These blotting media were tried with and without blocking with 5% BSA, or washing with 0.1% Triton X-100. In addition, binding by proteins dot blotted onto TFA-etched (trifluoroacetic acid) and unetched GFF was compared.

TFA etching of GFF

GFF was acid etched as described by Aebersold [10]. Briefly, the filters were carefully saturated with TFA in a Pyrex glass dish. After 1 h the TFA was removed, and filters were allowed to dry. Filters were stored in a glass container.

GFF incubation and protocol optimization

Several conditions for incubation were tested, including duration, pH of buffer, buffer type (Tris and KPO_4), temperature, and number of washes needed to remove free JH from the filter after incubation.

The optimal time for blot incubation was tested by dot blotting bovine serum albumin (background) or hemolymph (sample) on separate GFF disks and incubating with [10-^{3}H]JH-III and with or without unlabeled JH-III for 5, 15, and 30 min.

The optimal pH for incubation and washing was tested with 50 mM KPO_4 buffers at pH 6.0, 6.3, 6.7, 7.0 or 7.3; and 50 mM Tris–HCl buffers pH 7.3, 7.7 or 8.0 (NaCl content was 200 mM for Tris and PO_4 buffers).

To test the number of washes needed to remove free JH from filters without decreasing sample binding, we dot blotted BSA and hemolymph on separate disks of GFF and washed with TN buffer in the following regimens: two times for 5 min, four times for 5 min, four times for 5 min plus once for 20 min, and four times for 5 min plus four times for 20 min.

Two staining procedures for GFF were evaluated. Coomassie blue blot stain (30% isopropanol, 0.5% Coomassie R-250, 10% acetic acid; stain 2 min; destain for 10 min or longer in 16.2% methanol, 5% acetic acid [10]), and a modification of the Amido black stain by Schaffner and Weissmann (2 g Amido black, 500 ml methanol, 500 ml distilled H_2O, 100 ml acetic acid, stain for 10 min; destain in 5:5:1, methanol:distilled H_2O:acetic acid [13].

In the process of developing our dot blot procedure, we discovered that inks from felt tip permanent markers caused variable amounts of [10-^{3}H]JH-III to adhere to the filters.

Comparison of protein concentration with specific JH binding

Several dilutions of hemolymph were applied to GFF disks, the disks were incubated in [10-^{3}H]JH-III and specific binding was determined by scintillation spectroscopy. Protein concentration was determined by the Bio-Rad protein assay based on the method of Bradford [14] with bovine serum albumin as the standard protein.

Electroblotting

Polyacrylamide gels were blotted according to the following general procedure unless otherwise indicated. Following PAGE, gels were equilibrated for 15 min in 1.0% acetic acid and assembled into a blotting sandwich apparatus [10]. The blot was run

for 2 h at a constant voltage of 150 V, and approximately 600 mA. Following blotting, gels were stained in Coomassie R-250 stain for PAGE. Portions of blots were stained with Coomassie blot stain (see above), or cut into 1 × 1 or 1 × 2 cm pieces for incubation in [10-^{3}H]JH-III with or without unlabeled JH-III (Calbiochem, La Jolla, Calif.). Incubated pieces were either counted by scintillation spectroscopy as previously described [15] or dried and reassembled for fluorography. To prepare for fluorography, blots were sprayed three times with En^{3}Hance (New Engalnd Nuclear, Boston, Mass), and exposed to Kodak XAR 5 film at −70°C for 30 days or more depending on dpm bound.

RESULTS

Membrane selection

Of the four media tried for blotting, Zeta Probe and GFF had the lowest background after incubation in [10-^{3}H]JH-III. Nitrocellulose had the highest counts from non specific sticking of hormone to the medium, and blocking with 5% BSA, nonlabeled JH-III, or washing with Triton X-100 did not reduce the [10-^{3}H]JH-III background. Although Zeta Probe demonstrated low nonspecific sticking of hormone, proteins blotted on Zeta Probe did not bind JH (data not shown). Proteins blotted onto GFF did bind JH-III and binding was proportional to the amount of protein applied (Fig. 2). Furthermore, GFF did not require BSA blocking or washing with Triton X-100 to reduce background counts. Triton X-100 in the final washes reduced counts associated with specific binding (Fig. 3).

Comparison of etched and unetched GFF demonstrated no difference in the amount of binding when protein was blotted directly onto the medium. However, when etched and unetched GFF were compared in an electroblotting experiment, blotted proteins were found on the first sheet of etched GFF whereas proteins passed through the unetched GFF to the filter paper layers of the sandwich.

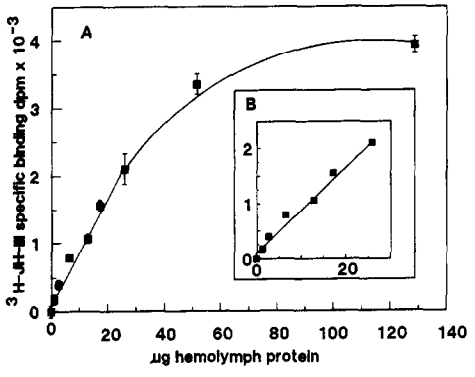

Fig. 2. Specific binding of juvenile hormone as a function of the amount of protein dot blotted onto GFF. Hemolymph samples of 20 µl of varying protein concentrations were applied to GFF disks. The disks were preincubated for 15 min in TN buffer, incubated for 15 min in either 10 nM [10-^{3}H]JH-III or 10 nM [10-^{3}H]JH-III plus 1 µM unlabeled JH-III, and washed in TN (four washes for 5 min). Disks were counted by scintillation spectroscopy. Protein concentration was determined by the Bradford method [14]. Part A shows specific binding for the entire range of protein applied to GFF. Part B shows a linear regression of the first seven points, the correlation coefficient $r = 0.99$.

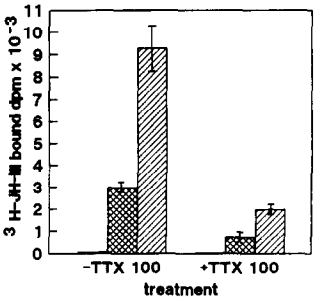

Fig. 3. The effect of Triton X-100 washes on background and protein bound [10-^{3}H]JH-III. Hemolymph from *M. bivittatus* was applied to GFF disks. Black is for buffer alone, crosshatching for 5.1 µg, and diagnonal lines for 10.1 µg of hemolymph protein. Disks were preincubated for 30 min in TN buffer, incubated 30 min in 10 nM [10-^{3}H]JH-III, and washed in TN plus 0.1% TTX-100 or in TN without TTX-100. The washing protocol included four washes for 5 min plus four washes for 20 min. Disks were dried and counted by scintillation spectroscopy.

GFF incubation

Experiments to determine the optimal time for blot incubation showed that specific JH binding sites were nearly filled after 15 min and binding increased by only 9% in the next 15 min (Fig. 4).

Determination of the optimal pH for incubation and washing showed that for the buffers tried in the range of pH 6.0–8.0, there was no significant difference in the amount of JH bound or in the background non specific association of JH to the disks (Fig. 5). Since none of the buffers were statistically better, we continued to use TN buffer.

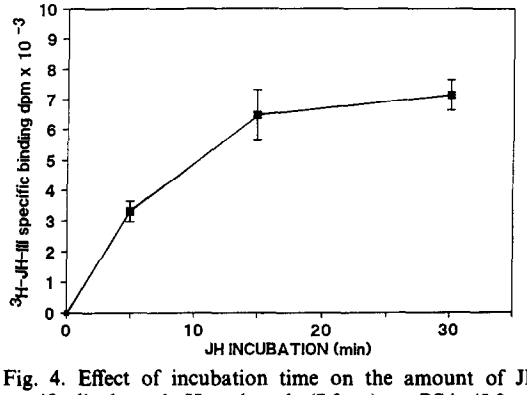

Fig. 4. Effect of incubation time on the amount of JH specifically bound. Hemolymph (7.0 µg) or BSA (5.0 µg) were applied to GFF disks. Disks were preincubated in TN for 15 min, incubated in 10 nM [10-^{3}H]JH-III only or 10 nM [10-^{3}H]JH-III plus 1 mM JH-III and washed in TN (four washes for 5 min). Disks were dried and counted by scintillation spectroscopy. No hormone was bound by BSA.

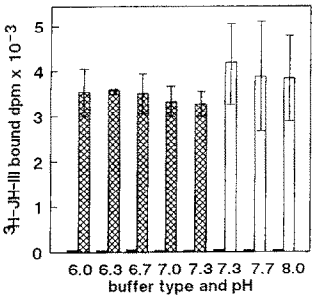

Fig. 5. The influence of buffer and pH on JH binding by hemolymph. Samples were diluted, washed and incubated in one of several phosphate or Tris buffers. All buffers contained 200 mM NaCl and were either 50 mM KPO_4 or 50 mM Tris. Phosphate buffers were adjusted to pH 6.0, 6.3, 6.7, 7.0 and 7.3. Tris buffers included pH 7.3, 7.7 and 8.0. Hemolymph (5.1 μg) or buffer was applied to GFF disks, the disks were preincubated for 15 min, incubated in 10 nM [10-^{3}H]JH-III for 15 min, washed four times for 5 min, dried and counted by scintillation spectroscopy.

Most [10-^{3}H]JH-III loosely associated with GFF or protein that does not bind JH (BSA) was removed by washing four times for 5 min. Binding by hemolymph stayed constant through the washing. Extremely long washing protocols (four times for 5 min plus four washes for 20 min) did not decrease the background but caused a 27% loss of [10-^{3}H]JH-III from the binding protein (Fig. 6).

Both the Coomassie and Amido black blot stains worked well on GFF although visualization of protein bands was slightly faster with the Coomassie stain.

Electroblotting

After electroblotting for 2 h approximately one-half to two-thirds of the protein in each band had transferred from a 1.5 mm gel (qualitative estimate after staining with Coomassie). Stained blots showed good transfer of protein bands, with little loss to a second sheet of GFF. Incubation of these back-up sheets showed that less than 0.5% of the binding protein passed through the first sheet of GFF. Fluorograms from native gradient and native mini PAGE blots showed three high molecular weight bands, 479, 331 and 295 k, which bind JH (Fig. 7). JH was affiliated with the 479 k band only on one fluorogram of five and on several electroblots analyzed only by scintillation spectroscopy. The two lower bands were always labeled, however, in initial

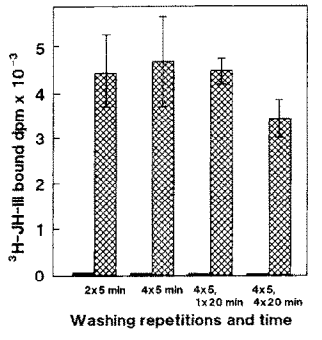

Fig. 6. The effect of washing duration and repetition on background and protein bound [10-^{3}H]JH-III. Hemolymph protein from (5.1 μg) or BSA (5.0 μg) was dot blotted onto GFF. Disks were preincubated for 30 min in TN buffer and incubated in 10 nM [10-^{3}H]JH-III for 30 min. Washes differed in number and duration, the protocols used were: two times for 5 min, four times for 5 min, four times for 5 min plus one time for 20 min, or four times for 5 min plus four times for 20 min. Disks were dried, and counted by scintillation spectroscopy.

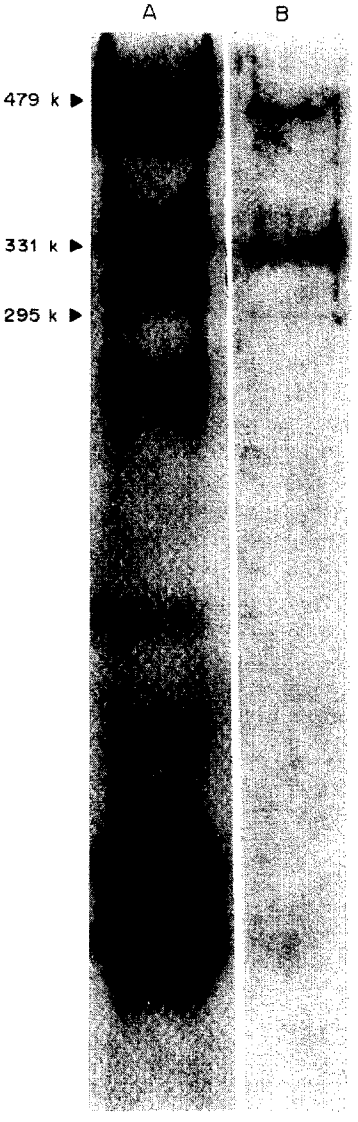

Fig. 7. Fluorogram of an electroblot of a native gradient gel. Hemolymph was applied to native gradient PAGE 5–18% run 10 h at 160 V. The gel was blotted for 2 h at 150 V, 620 mA. One portion of the blot was stained for 2 min with Coomassie blue blot stain (A), another portion was incubated in 10 nM [10-^{3}H]JH-III in TN buffer and washed four times 5 min. One channel was counted by scintillation spectroscopy, the adjacent channel was sprayed with En^{3}Hance (New England Nuclear) for fluorography (B). The X-ray film was exposed to the blot for 90 days at −70°C. Determination of M_rs was by Ferguson analysis (data not shown).

experiments, 331 and 295 k were so close that they could not be discriminated as two bands. Gradient gels separated the 331 k, an intermediate band, and 295 k bands on stained gels. In this region, the 331 and 295 k bound JH. The 295 k band was a very minor band on the gel shown in Fig. 7, and constituted no more than 2% of the total protein on any gel by Coomassie staining. Binding in the region of the JH-binding bands was decreased by addition of unlabeled JH in competition experiments. Duplicate lanes of several electroblots of hemolymph proteins were incubated in [10-^{3}H]JH-III with or without unlabeled JH-III. By scintillation spectroscopy, the binding was decreased to background when unlabeled JH was present.

DISCUSSION

This is the first report of detection of electroblotted hormone-binding proteins by incubating blots with radiolabeled ligands of the steroid-terpenoid class of hormones. Such an application of electroblotting was suggested in 1979 by Towbin, who predicted that it would be used for detection of a variety of molecules including hormone receptors [16]. Although several proteins have been detected by ligands bound after blotting, prior to this report the only hormones used to locate their binders have been peptides [17–19]. Identification of individual hormone-binding proteins is critical for understanding the complexity of lipid hormone delivery to target cells and subsequent action. The advantages of identifying proteins after electrophoresis are multifold. Labeling proteins after PAGE circumvents the problems of dissociation of non-covalently bound hormone which were indicated in the introduction. In addition, the native hormone rather than a synthetic analog can be used to detect binding proteins. Synthetic photoaffinity ligands may have biologically significant modifications to allow light-activated covalent binding.

The techniques for blotting are also simpler than those used with photoaffinity labels or other analogs thus far reported in the literature. To detect tritium in gels one must either cut out the protein band and digest the gel for scintillation spectroscopy, or embed the gel for fluorography. Embedding gels for fluorography renders [10-^{3}H]EFDA labeled proteins unusable for blotting and microsequencing. Although gels with [12-^{125}I]JH-labeled samples do not require embedding, preparation for microsequencing is complex. Gels must be soaked, dried, exposed to X-ray film, rehydrated, and transferred to GFF [20]. The simplicity of our method compared to this is clear.

The type of medium used also contributed to the ease of analysis. With nitrocellulose, no amount of blocking or washing would reduce background binding of [10-^{3}H]JH-III. Only GFF allowed high binding with low background counts. Low background adsorption of JH-III on GFF as compared to nitrocellulose may reflect differences in chemical properties of these membranes. Aebersold speculates that the mechanism of protein adsorption on GFF is ionic interaction [10], whereas hydrophobic forces have been implicated in retaining protein on nitrocellulose [17]. Since JH is a terpenoid, hydrophobic forces may cause JH to adhere to nitrocellulose, whereas JH may not absorb to GFF because of its negatively charged sites.

GFF afforded additional advantages as the medium. It can be used directly for scintillation counting with no digestion (Jefferies and Roberts, unpublished data), GFF allows detection of BPs by hormone binding and by antibodies, and proteins on GFF can be directly inserted into a gas phase sequenator or can be removed easily with solvents or detergents [10]. In comparison to nitrocellulose, GFF is less brittle and easier to use and, unlike nitrocellulose [12], does not tend to lose small proteins during washing (data not shown).

Although equal amounts of JH were bound by proteins dot-blotted on TFA etched and unetched GFF, results from electroblotting showed that etched GFF was superior in retaining protein. In dot blotting, proteins are applied to the surface of the GFF, whereas electroblotted proteins are subjected to the strong forces of electrical transfer and must be retained by the GFF.

The conditions for selective binding of JH by blotted proteins were determined. Incubation for 15 min was sufficient for JH to be bound by proteins. Longer incubations did yield small increases in binding, but the procedure was intended for locating of JHBPs rather than quantitation. Two types of buffers and a wide pH range were tried for this assay. The levels of binding were the same in all PO_4 and Tris buffers. In all cases, what appeared to be slightly higher binding with Tris buffer was not statistically significant, and the standard deviation was greater for Tris than for PO_4.

After incubation in JH-III, we found that washing four times for 5 min was adequate for removal of background counts. Extremely long washing protocols resulted in loss of protein bound JH. Although it is not clear whether we removed only JH or the protein-hormone complex, excessive washing eliminated more than unbound hormone and was unnecessary.

A number of staining methods can be used effectively to detect the protein bands on the blots. The Coomassie method was selected because it stained quickly with high sensitivity, and it allowed rapid evaluation of protein transfer before continuing with incubation in hormone.

By using the electroblot method, we detected three bands which bind JH. As indicated in the results, the 479 k band was detected only on one fluorogram. Inconsistent labeling of this band suggests that it may be a non-specific binding protein, but physiological differences in the insects from which the hemolymph samples were taken might be another explanation.

JH-binding proteins labeled with [10-^{3}H]JH-III after electroblotting may differ from those detected by other methods or by analogs. Earlier separations of *M. bivittatus* hemolymph on DEAE cellulose followed by Sephadex G150 showed JH-binding proteins of 10, 26, 52, 106 and 125 k [11]. Compared to these earlier data, the M_rs for hemolymph proteins reported in this paper (M_rs 479, 331 and 295 k) are high, however high molecular weight complexes for JHBPs have been noted in a number of species [21]. In *M. bivittatus*, EFDA shares binding sites with JH on the same three BPs detected on blots from native gels plus an additional band at 54 k. These three high molecular weight bands were resolved into subunits of 240, 77 and 72 k on SDS gels, however the number of peptides labeled with EFDA and degree of labeling were quite variable between experiments [2].

The lack of uniformity in the molecular weights reported for the JH binders in *L. migratoria* hemolymph is a good example of the variable results obtained when different methods are used. de Bruijn [21] reports that the locust JHBP has a M_r of 575 k and is a hexamer of 81 k subunits. These values are close, but not identical to the locust hemolymph proteins photolabeled with [10-^{3}H]JH-III which have a M_r of 450 k after separation by HPLC and subunits of 90 k on SDS gels [1]. In contrast, Emmerich and Hartmann report that labeling locusts *in vivo* results in JH binding by a single hemolymph protein of 220 k [22]. Clearly, these differences need to be resolved.

In other insects, only a single peptide binding JH has been found by electrophoretic separation of proteins photolabeled with [10-^{3}H]JH or with [10-^{3}H]-EFDA [4, 5, 23]. In most cases, EFDA binds to several proteins, but is only competitively displaced by JH from one or two bands [3, 4, 6, 24]. EFDA binding without displacement by JH has been interpreted as EFDA association with non-specific sites, but data to substantiate this claim are weak or absent and need clarification.

There are varying characteristics reported regarding the nature of the JHBPs. Of the insects which have only lipophorins as JH binders, the apolipophorins have molecular weights of about 245 and 78 k. Koeppe [25, 26] concludes that *Leucophaea maderae* JHBP is a lipophorin based on molecular weight alone.

In contrast, de Bruijn *et al.* (1986) have carefully identified the hemolymph proteins of *L. migratoria*, and show that the JHBP differs from lipophorin, cyanoprotein, and larval storage protein. The only lipophorin in the hemolymph of *M. bivittatus* detected by Sudan black B gel staining had a M_r of 479 k (Li and Roberts, unpublished data). By the electroblot method, we also detected a 479 k protein which binds JH although we have detected JH binding only on one electroblot. The other two JH binding proteins (331 and 295 k) do not stain with Sudan black B.

We have shown that JHBPs from *M. bivittatus* may be electroblotted onto TFA etched GFF. Proteins on the blot retain their ability to bind [10-^{3}H]JH specifically, and in this manner, binding proteins can be identified by scintillation spectroscopy and fluorography. Preliminary results from dot blotting experiments suggest that estrogen binding proteins may be similarly identified on GFF (data not shown).

Acknowledgements—We thank Yuchang Li for her assistance with PAGE separation of proteins, and Dr Bruce Winder for his information on EFDA and photolabeling proteins. This research was supported by NIH grant AI-18470-1-01AI TMP and NSF DCB-8710056 to P.E.R. Preliminary studies were supported by Biomedical Research Support Grants and Faculty Research Grants through Colorado State University.

REFERENCES

1. Stupp H. P. and Peter M. G.: Juvenile hormone III as a natural ligand for photoaffinity labeling of JH-binding proteins. *Z. Naturforsch.* **39c** (1984) 1145–1149.
2. Winder B. S.: Juvenile hormone-binding proteins of *Melanoplus bivittatus* identified by EFDA photoaffinity labeling. PhD dissertation, Colorado State University (1988).
3. Prestwich G. D., Robles S., Wawrzenczyk C. and Buhler A.: Hemolymph juvenile hormone binding proteins of lepidopterous larvae: enantiomeric selectivity and photoaffinity labeling. *Insect Biochem.* **17** (1987) 551–560.
4. Prestwich G. D., Koeppe J. K., Kovalick G. E., Brown J. J., Chang E. S. and Singh A. K.: Experimental techniques for photoaffinity labeling of juvenile hormone binding proteins of insects with epoxyfarnesyl diazoacetate. In *Methods in Enzymology* (Edited by J. H. Law and H. C. Rilling). Academic Press, Orlando, Vol. 111 part B (1985) p. 509.
5. Koeppe J. K., Prestwich G. D., Brown W. G., Goodman W. G., Kovalick G. E., Briers T., Pak M. D. and Gilbert L. I.: Photoaffinity labeling of the hemolymph juvenile hormone binding protein of *Manduca sexta*. *Biochemistry* **23** (1984) 6674–6679.
6. Koeppe J. K. and Kovalick G. E.: A specific photoaffinity label for hemolymph and ovarian juvenile hormone-binding proteins in *Leucophaea maderae*. *J. Biol. Chem.* **259** (1984) 3219–3223.
7. Roberts P. E.: Characterization of juvenile hormone-binding proteins of hemolymph of *Locusta migratoria*. *Arch. Insect Biochem. Physiol.* **2** (1985) 351–365.
8. Riddiford L. M., Osir E. O., Fittinghoff C. M. and Green J. M.: Juvenile hormone analog binding in *Manduca* epidermis. *Insect Biochem.* **17** (1987) 1039–1043.
9. Mohamed M. and Prestwich G. D.: Hemolymph juvenile hormone binding proteins of *Reticulitermes flavipes*: induction by methoprene and suppression by pheromones. In *Endocrinology Frontiers of Physiological Insect Ecology* (Edited by F. Senal and A. Zabza). International Conference in Sklarska-Poreba, Poland (1987) In press.
10. Aebersold R. H., Teplow D. B., Hood L. E. and Kent S. B. H.: Electroblotting onto activated glass: high efficiency preparation of proteins form analytical sodium dodecyl sulfate-polyacrylamide gels for direct sequence analysis. *J. Biol. Chem.* **261** (1986) 4229–4238.
11. Roberts P. E. and Jefferies L. S.: Grasshopper as a model system for the analysis of juvenile hormone

delivery to chromatin acceptor sites. *Arch. Insect Biochem. Physiol.* (Suppl. 1) (1986) 7–23.
12. Jahn R., Shiebler W. and Greengard P.: A quantitative dot-immunobinding assay for proteins using nitrocellulose membrane filters. *Proc. Natn. Acad. Sci. U.S.A.* **81** (1984) 1684–1687.
13. Schaffner W. and Weissmann C.: A rapid, sensitive, and specific method for the determination of protein in dilute solution. *Analyt. Biochem.* **56** (1973) 502–514.
14. Bradford, M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72** (1976) 248–254.
15. Roberts P. E. and Wyatt G. R.: Juvenile hormone binding by components of fat body cytosol from vitellogenic locusts. *Molec. Cell. Endocr.* **31** (1983) 53–69.
16. Towbin H., Staehelin T. and Gordon J.: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natn. Acad. Sci. U.S.A.* **76** (1979) 4350–4354.
17. Towbin H. and Gordon J.: Immunoblotting and dot immunobinding-current status and outlook. *J. Immun. Meth.* **72** (1984) 313–340.
18. Haeuptle M. T., Aubert M. L., Djiane J. and Kraehenbuhl J. P.: Binding sites for lactogenic and somatogenic hormones from rabbit mammary gland and liver: their purification by affinity chromatography and their identification by immunoprecipitation and photoaffinity labeling. *J. Biol. Chem.* **258** (1982) 305–314.
19. Islam M. N., Briones-Urbina R., Bako G. and Farid, N. R.: Both TSH and thyroid-stimulating antibody of Graves' disease bind to an Mr-197,000 holoreceptor. *Endocrinology* **113** (1983) 436–438.
20. Kulcsar P. and Prestwich G. D.: Detection and microsequencing of juvenile hormone-binding proteins of an insect by the use of an iodinated juvenile hormone analog. *FEBS Lett.* **228** (1987) 49–52.
21. deBruijn A. M., Koopmanschap A. B. and deKort C. A. D.: High-molecular-weight serum proteins from *Locusta migratoria*: identification of a protein specifically binding juvenile hormone III. *Physiol. Entomol.* **11** (1986) 7–16.
22. Emmerich H. and Hartmann R.: A carrier lipoprotein for juvenile hormone in the haemolymph of *Locusta migratoria*. *J. Insect Physiol.* **19** (1973) 1663–1675.
23. de Kort C. A. D. and Koopmanschap A. B.: Molecular characteristics of lipophorin, the juvenile hormone-binding protein in the hemolymph of the Colorado potato beetle. *Arch. Insect Biochem. Physiol.* **5** (1987) 255–269.
24. Shemshedini L. and Wilson T. G.: A high affinity, high molecular weight juvenile hormone binding protein in the hemolymph of *Drosophila melanogaster*. *Insect Biochem.* **18** (1988) 681–689.
25. Koeppe J. K., Rayne R. C., Shearin M. D., Carver D. J., Whitsel E. A. and Vogler R. C.: Synthesis and secretion of a precursor hemolymph juvenile hormone-binding protein in the adult female cockroach *Leucophaea maderae*. *Insect Biochem.* **18** (1988) 661–667.
26. Rayne R. C. and Koeppe J. K.: Relationship of hemolymph juvenile hormone-binding protein to lipophorin in *Leucophaea maderae*. *Insect Biochem.* **18** (1988) 667–673.