Pergamon

PII: S0965-1748(97)00042-8

Insect Biochem. Molec. Biol. Vol. 27, No. 7, pp. 663–670, 1997

0965-1748/97 $17.00 + 0.00

Lipophorin: A Hemolymph Juvenile Hormone Binding Protein in the German Cockroach, *Blattella germanica*

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Received 14 January 1997; revised and accepted 4 June 1997

We examined the binding of [^{3}H](10*R*) juvenile hormone (JH) III to lipophorin that was purified from the hemolymph of *Blattella germanica*. Binding was found to be specific, saturable and with high affinity to JH III. Using Scatchard analysis, the equilibrium dissociation constant (K_d) and total binding capacity (B_{max}) were estimated to be 9.75 ± 0.64 nM and 0.241 ± 0.02 nmol/mg protein, respectively. Competitive displacement studies with racemic JH III, JH I, cuticular hydrocarbon, contact sex pheromone, and the JH analogs pyriproxyfen, fenoxycarb, and hydroprene showed that only JH III readily displaced [^{3}H](10*R*)JH III from the binding site. However, hydroprene competed for the JH III binding site more effectively than the other two JH analogs. Photoaffinity labelling using the JH III analog [^{3}H]epoxyfarnesyl diazoacetate demonstrated that the JH binding site was on apolipophorin-I, the large subunit of the lipophorin complex. © 1997 Elsevier Science Ltd

Blattella germanica Lipophorin Juvenile hormone Binding protein Hemolymph Pyriproxyfen Fenoxycarb Pheromone Photoactivation

INTRODUCTION

Juvenile hormones (JH) play important roles in insect metamorphosis and reproduction. Juvenile hormones are hydrophobic (solubility limit of 54 μM in aqueous buffer; Trowell, 1992), and it has been well established from various studies that JH is transported from the corpora allata (CA) to target tissues by plasma JH binding proteins (JHBP) (Goodman, 1990; Trowell, 1992). In *Manduca sexta* virtually all JH in hemolymph exists as a complex with JHBP (Hidayat and Goodman, 1994).

Whitmore and Gilbert (1972) first reported the occurrence of a JHBP in the hemolymph of *Hyalophora gloveri*. Since then, several such proteins have been isolated and characterized from a number of insect taxa (Goodman and Chang, 1985; Trowell, 1992; Prestwich *et al*., 1994). On the basis of their molecular weight and affinity to JH, three different types of JHBPs have been identified. In *M. sexta* and other Lepidoptera, low molecular weight JHBPs (M_r ~ 30 kDa) have been shown to have relatively high affinity (dissociation constant 72–650 nM) to JH I and JH II (for review see Goodman and Chang, 1985; Trowell, 1992). The other two types are high molecular weight lipoproteins. One is a very high density lipoprotein (d = 1.25 mg/ml) that functions as a JH III transporter in *Locusta migratoria*; it is composed of six identical subunits (77 kDa), each of which contains a JH binding site (Koopmanschap and de Kort, 1988). The other is lipophorin (Lp), a high density lipoprotein which has a single site that binds JH III with high specificity and high affinity (K_d for racemic JH III = 1.5 to 157 nM). This type of JHBP has been isolated from several insects, namely, Coleoptera (*Leptinotarsa decemlineata*; de Kort and Koopmanschap, 1987), Isoptera (*Reticulitermes flavipes*; Okot-Kotber and Prestwich, 1991), Diptera (*Sarcophaga bullata*; Van Mellaert *et al*., 1985; *Drosophila melanogaster*; Shemshedini and Wilson, 1988; *Chironomus thummi*; Wisniewski and Streuernagel, 1990), Hymenoptera (*Apis mellifera*; de Kort and Koopmanschap, 1986), and Dictyoptera (*Periplaneta americana*; de Kort and Koopmanschap, 1986; *Leucophaea maderae*; Rayne and Koeppe, 1988; *Nauphoeta cinerea*; Kindle *et al*., 1989; *Diploptera punctata*; King and Tobe, 1988). Structural studies of lipophorin indicate that the holoprotein consists of two apoproteins, apolipoprotein-I (apoLp-I; M_r ~ 250 kDa) and apolipoprotein-II (apoLp-II; M_r ~ 85 kDa) (for reviews see Kanost *et al*.,

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1990; Law *et al*., 1992; Van der Horst *et al*., 1993; Soulages and Wells, 1994). A distinct, diapause-associated high molecular weight JHBP has been isolated from a single lepidopteran, *Busseola fusca* (Osir *et al*., 1991). Interestingly, both Lp and a low molecular weight protein serve as JH binding proteins in the midge *Chironomus thummi* (Wisniewski and Streuernagel, 1990).

A number of reports conclude that, in addition to its transport function, JHBP protects JH from degradation by hemolymph esterolytic enzymes (see Goodman, 1990; Trowell, 1992). However, recent evidence suggests that in *M. sexta*, JH esterases interact with JHBP, causing release of JH from the binding pocket and faster hydrolysis; yet, JHBP appears to protect JH from JH epoxide hydrolases (Touhara *et al*., 1996). It is still unknown whether lipophorin-type JHBPs, which have higher affinity to the hormone, more effectively protect JH, as is suggested for cockroaches (Engelmann *et al*., 1988; Lanzrein *et al*., 1993). In either case, JHBPs appear to play key roles in JH transport and the regulation of JH titer.

In *Blattella germanica*, lipophorin is a multi-functional lipid carrier. It is involved in the transport of hydrocarbon and pheromone to the epicuticle (Gu *et al*., 1995), and is rich in diacylglecerol and phospholipid (unpublished). However, it is not known whether lipophorin functions as a JH carrier in *B. germanica*. In this paper we report binding properties of a purified JH binding lipophorin.

MATERIALS AND METHODS

Chemicals

Unless otherwise indicated, all chemicals were purchased from Sigma (St Louis, MO, U.S.A.). [^{3}H](10*R*,11*S*) Epoxyfarnesyl diazoacetate (EFDA) (specific activity 5.4 Ci/mmol), a photoaffinity analog of JH III, was a gift from Dr G. D. Prestwich (University of Utah, Salt Lake City, UT, U.S.A.). Natural [^{3}H](10*R*)JH III was biosynthesized in our laboratory. L-[Methyl-^{3}H]methionine (71.4 Ci/mmol) was obtained from Dupont–NEN (Wilmington, DE, U.S.A.) and diluted to 14.28 Ci/mmol prior to use. 3-Octylthio-1,1,1-trifluoropropan-2-one (OTFP), an inhibitor of JH esterase, was a gift from Dr R. M. Roe (North Carolina State University, Raleigh, NC, U.S.A.). Juvenile hormone analogs were supplied as follows: fenoxycarb was a gift from Dr R. Maag Ltd. (now part of Novartis, Greensboro, NC, U.S.A.), pyriproxyfen was obtained from Zeneca (Wilmington, DE, U.S.A.) and (7*S*)hydroprene from Sandoz (Palo Alto, CA; now part of Novartis). Hydrocarbons from *B. germanica* were extracted from the cuticle of adult females and their mass determined by gas-liquid chromatography relative to a *n*-heptacosane internal standard (Schal *et al*., 1994). The contact pheromone 3,11-dimethylnonacosan-2-one (a synthetic racemic mix) was a gift from Dr R. Nishida (Kyoto University, Kyoto, Japan).

Insects

German cockroaches (*B. germanica*) were maintained in an incubator at 27 ± 0.3°C under a 12 h light:dark photoregime and were allowed free access to Purina rat chow and water. Only virgin adult females were used in the experiments.

Hemolymph collection

Hemolymph was collected from CO_2-anesthetized insects by severing the forelegs and applying gentle pressure to the abdomen. Hemolymph was collected into chilled 1.5 ml microcentrifuge tubes and centrifuged at 6000***g*** at 4°C for 8 min to pellet hemocytes. The plasma was stored at –80°C until use.

Biosynthesis of [^{3}H](10R)JH III

Corpora allata from 4-day-old mated female *D. punctata* were dissected and incubated *in vitro* in L15B medium (Holbrook *et al*., 1997) containing 10^{-5} M OTFP, a JH esterase inhibitor, and [^{3}H]methionine for 6 h at 27°C. Labelled JH was extracted twice with isooctane and stored at –80°C. The purity of radio-biosynthesized JH was checked on a Beckman high pressure liquid chromatograph (Beckman Instruments, Fullerton, CA, U.S.A.) equipped with model 125 pumps, a model 166 UV detector and a model 171 radioactivity flow detector. Isooctane extracts were re-constituted in acetonitrile and chromatographed on a Hibar C_{18} reverse-phase column (4.6 mm × 25 cm, 5 μm particle size) for 50 min in a linear gradient of 40–100% acetonitrile in 5 mM HEPES buffer (pH 7.5) at 1 ml/min. Scintillation fluid (Beckman Ready-Flow III) flowrate was 3 ml/min. Total flow through the radiodetector was 4 ml/min.

Isolation and purification of lipophorin by gradient ultracentrifugation

Lipophorin was purified by KBr density-gradient ultracentrifugation as described by Shapiro *et al*. (1984) and previously applied to *B. germanica* by Gu *et al*. (1995). Hemolymph (400 μl) obtained from 4-day-old virgin females was subjected to ultracentrifugation at 285,000***g*** for 22 h, at 4°C in a Beckman L8-70M ultracentrifuge using a fixed angle rotor (70.1 Ti). Fractions were collected starting from the top of the tube. The purity of lipophorin fractions was checked by native and sodium dodecyl sulfate (SDS) gel electrophoresis.

Juvenile hormone binding assay

All glassware used in JH binding and photoaffinity labelling experiments was precoated with 1% polyethylene glycol (PEG-M_r 20,000) as described by Sevala *et al*. (1995). JH binding assays were carried out by using the procedure of Koeppe *et al*. (1981) as modified by King and Tobe (1988). JH binding was estimated in glass borosilicate tubes (6 × 50 mm) containing 200 μl aliquots of purified lipophorin (5 μg/ml) in TMSG buffer (10 mM Tris, 5 mM $MgCl_2$, 150 mM NaCl, pH 7.4, and 0.5 mg

globulin) and 10 nM [^{3}H](10*R*)JH III. After 30 min incubation at 27°C, the bound ligand/protein complex was precipitated with 32% PEG (M_r 6000, in TMS buffer) and radioactivity was measured in the pelleted precipitate and supernatant with a Beckman 5801 liquid scintillation spectrometer. Radioactivity in the precipitate represented JHBP-bound JH, whereas radioactivity in the supernatant represented free ligand. Non-specific binding was determined by the addition of 100-fold excess unlabelled ligand to an identical set of samples prior to the incubation. Specific binding was determined by subtracting non-specific binding from total binding. The kinetic constants were estimated by Scatchard analysis (Scatchard, 1949).

We also tested whether lipids that are known to bind lipophorin (hydrocarbon and contact sex pheromone (Gu *et al*., 1995)) and JH analogs (fenoxycarb, pyriproxyfen, and hydroprene) could competitively displace [^{3}H](10*R*)JH III from JH binding sites. An aliquot of 10 nM [^{3}H](10*R*)JH III was incubated with purified lipophorin in the presence of different concentrations of these ligands. The amount of bound [^{3}H](10*R*)JH III was determined by PEG binding assay.

Photoaffinity labelling

Photoaffinity labelling of lipophorin was performed according to Prestwich (1991). Aliquots of purified lipophorin (6μg/100 μl) were incubated with 200 nM [^{3}H]EFDA at 4°C in the presence or absence of 200-fold excess unlabelled racemic JH III in quartz tubes precoated with PEG (M_r 20,000). After 2 h, the samples were photolysed four times for 15 s each at a distance of 11 cm using a Stratagene UV crosslinker (Stratalinker Model 1800; La Jolla, CA, U.S.A.) set at maximum energy. Samples were lyophilized and resolubilized in sample buffer and subjected to native and SDS gel electrophoresis.

Gel electrophoresis and fluorography

Following photolysis, the photolabelled samples were separated by native and SDS–polyacrylamide gel electrophoresis (PAGE) using 4–12% gradient slab gels, according to the procedure of Laemmli (1970). Gels were stained with Coomassie blue to detect proteins, and were impregnated with 10% 2,5-diphenyloxazole in acetic acid. Gels were then dried and exposed to Kodak X-Omat AR X-ray film for 3 weeks at –80°C.

Protein determination

The protein concentration in hemolymph and lipophorin samples was determined using the BioRad protein assay (Bradford, 1976) with bovine serum albumin as a standard.

Statistical analysis

Data were subjected to Scatchard analysis (Scatchard, 1949) to determine the kinetic constants, namely the equilibrium dissociation constant (K_d) and total binding capacity (B_{max}). Linear regression was used to determine the best fit line to the experimental data. The statistical significance of difference between means was estimated by Student's *t*-test.

RESULTS

Earlier work in this laboratory has shown that lipophorin is composed of two apoproteins, apoLp-I and apoLp-II, and has a density of 1.109 g/ml; it was therefore designated a high density lipophorin (HDLp) (Gu *et al*., 1995). Preliminary binding studies indicated that [^{3}H](10*R*)JH III bound to a lipoprotein with similar density. Therefore, we have used purified lipophorin in all of our JHBP characterization studies. We re-examined the apoprotein composition of lipophorin. Native PAGE indicated a molecular weight for the native protein of 670 kDa. The two apoLp subunits, apoLp-I and apoLp-II, were 212 and 80 kDa, respectively. These results differ somewhat from previous determinations in our laboratory (Gu *et al*., 1995); the apoprotein composition of *B. germanica* lipophorin is thus similar to that of other insects.

Biosynthesis of [^{3}H](10R)JH III

Enantiomerically pure [^{3}H](10*R*)JH III was biosynthesized by *D. punctata* CA incubated *in vitro* with [^{3}H]methionine. Reverse-phase high pressure liquid chromatography (HPLC) indicated that 98.5% of the radioactivity in the isooctane hyperphase of the incubation medium coeluted with authentic JH III (Fig. 1). In all subsequent studies [^{3}H](10*R*)JH III was obtained from isooctane extracts of CA incubation medium without further purification.

Characterization of juvenile hormone binding site

In order to optimize conditions for the binding assays, the specific binding of JH to lipophorin was determined

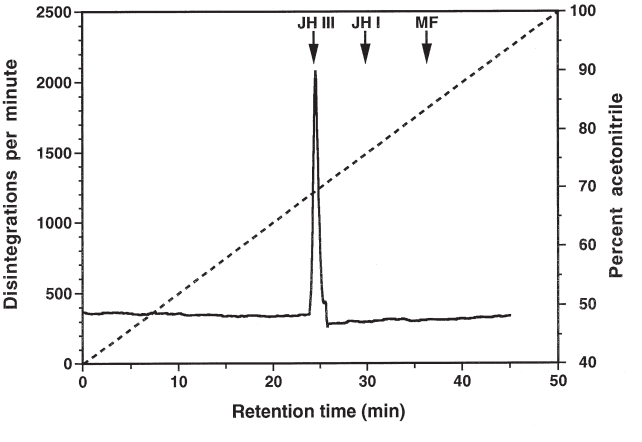

FIGURE 1. Reverse-phase high pressure liquid radio-chromatogram of *D. punctata* CA products. After a 6 h CA incubation in the presence of [^{3}H]methionine the incubation medium was extracted twice with isooctane, concentrated, and injected into the radio-HPLC. The dashed line indicates percentage acetonitrile in the mobile phase. JH I, Juvenile hormone I; JH III, juvenile hormone III; MF, methyl farnesoate.

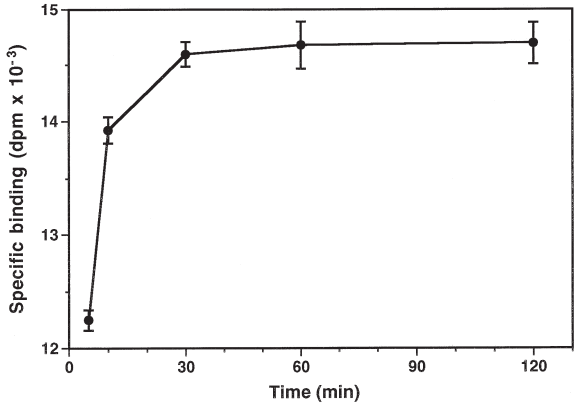

FIGURE 2. Time-course of [^{3}H](10*R*)JH III specific-binding to lipophorin. Potassium bromide (KBr) gradient-purified lipophorin (5 μg/ml) was incubated in 200 μl buffer with 10 nM [^{3}H](10*R*)JH III, in the presence or absence of 100-fold unlabelled racemic JH III for various time periods at 27°C and assayed for specific binding. Values represent the means of three replicates ± SEM.

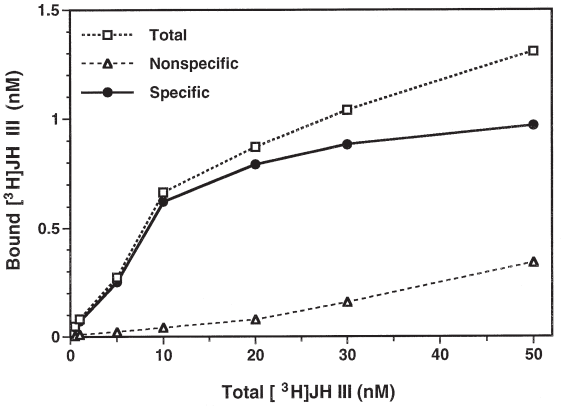

FIGURE 4. Specific binding of JH III by lipophorin. Purified lipophorin (1 μg) was incubated with various concentrations of [^{3}H](10*R*)JH III for 30 min at 27°C and assayed for specific binding. Non-specific binding was measured in the presence of 100-fold excess unlabelled racemic JH III. Each point is the mean of three replicates.

as a function of time and protein concentration. Fig. 2 shows the time-course of specific binding of [^{3}H](10*R*)JH III to lipophorin. The amount of hormone bound increased with incubation time and reached an apparent equilibrium after approximately 30 min at 27°C. In all subsequent experiments incubations were carried out for 30 min.

The relationship between specific binding and lipophorin concentration was also examined. JH III-specific binding was found to be proportional to the amount of lipophorin up to 5 μg per 0.2 ml incubation medium (Fig. 3).

The kinetic parameters of the JH binding site were deduced by incubating increasing concentrations of [^{3}H](10*R*)JH III with and without 100-fold excess unlabelled racemic JH III. The difference between total [^{3}H]JH III binding in the absence of unlabelled JH III and non-specific [^{3}H]JH III binding in the presence of unlabelled JH gave a measure of the specific binding. Fig. 4 shows that the specific binding increased with increasing concentration of [^{3}H](10*R*)JH III and demonstrated saturability at approximately 10 nM of [^{3}H](10*R*)JH III. Non-specific binding consisted of less than 25.9% of the total binding, and was found to be linear over the range of hormone concentrations tested. Scatchard transformation of the data followed by linear regression analysis indicated the presence of a single high affinity binding site with a dissociation constant (K_d) of 9.75 ± 0.64 nM (standard error of the mean) and a maximum binding capacity (B_{max}) at equilibrium of 0.241 ± 0.02 nmol/mg of lipophorin (Fig. 5).

Specificity of [^{3}H](10R)JH III binding

Hydrocarbon and contact sex pheromone are natural ligands of lipophorin in adult female *B. germanica* (Gu *et al*., 1995). These lipids, as well as JH I and JH analogs were tested for their ability to compete for the JH III binding site. Various concentrations of unlabelled

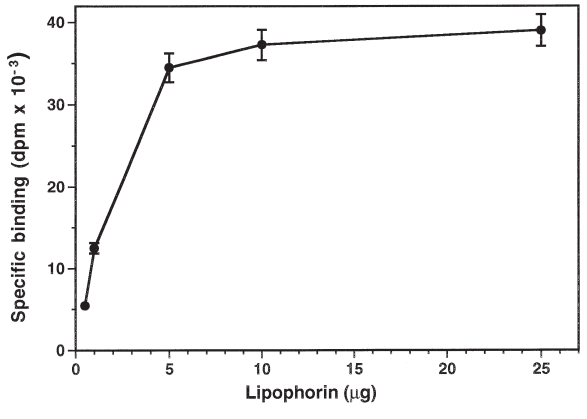

FIGURE 3. Specific binding of [^{3}H](10*R*)JH III to lipophorin as a function of lipophorin concentration. Various concentrations of purified lipophorin were incubated with 10 nM [^{3}H](10*R*)JH III at 27°C for 30 min in the presence or absence of 100-fold unlabelled racemic JH III. Data points represent the average of three separate determinations and vertical bars represent ± SEM.

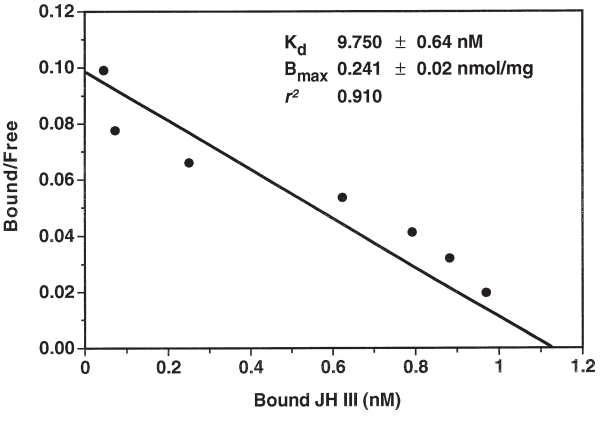

FIGURE 5. Scatchard plot of JH III-specific binding by lipophorin. The dissociation constant (K_d) was determined from the slope of the regression line ($-1/K_d$) and total binding capacity (B_{max}) was derived from the intercept of the line with the abscissa. The line was fitted by linear regression; r^2 is Pearson's correlation coefficient.

racemic JH III (10^{-5}–10^{-10} M) competed very effectively with [^{3}H](10*R*)JH III, showing 50% displacement at 10^{-7} M (Fig. 6). JH I, a higher homolog not found in the hemolymph of the German cockroach, was a much less effective competitor for the JH III binding site. Neither natural ligands (hydrocarbon and pheromone) nor synthetic analogs of JH III had significant effects on [^{3}H](10*R*)JH III binding to lipophorin. Interestingly, however, hydroprene, which shares a sesquiterpenoid structure with JH III, competed with [^{3}H](10*R*)JH III for the JH binding site more effectively than did other JH analogs that have structures unrelated to natural JH.

Photoaffinity labelling

To test whether the JH III binding site is present on apoLp-I or apoLp-II, [^{3}H]EFDA, a photoaffinity analog of JH III, was employed in our study. It was clear that native lipophprin (M_r 670 kDa) was covalently modified by [^{3}H]EFDA in the absence of competing ligand (Fig. 7). Competitive displacement of the photoactive ligand with 200-fold excess unlabelled racemic JH III greatly reduced [^{3}H]EFDA binding to lipophorin (Fig. 7). Denaturing SDS–PAGE indicated that only apoLp-I (212 kDa) and not apoLp-II (80 kDa) bound [^{3}H]EFDA (Fig. 8). Only apoLp-I thus has a binding site for JH III. [^{3}H]EFDA labelling of hemolymph confirmed that excess unlabelled JH III inhibited the binding of the photolabel to lipophorin only (data not shown).

DISCUSSION

Potassium bromide density gradient ultracentrifugation has been used extensively to purify lipophorin from insects. However, its utility in isolating and purifying JHBPs has been minimal. Using this technique Gu *et al*. (1995) isolated a high density lipophorin (density of 1.109 gm/ml) from the hemolymph of *B. germanica* and determined that it played a major role in the transport of cuticular hydrocarbons and contact pheromone. Its constituent apoproteins, apoLp-I and apoLp-II, have relative molecular weights of 212 and 80 kDa, respectively. In the present study we demonstrate that this lipophorin also functions as a plasma juvenile hormone binding protein.

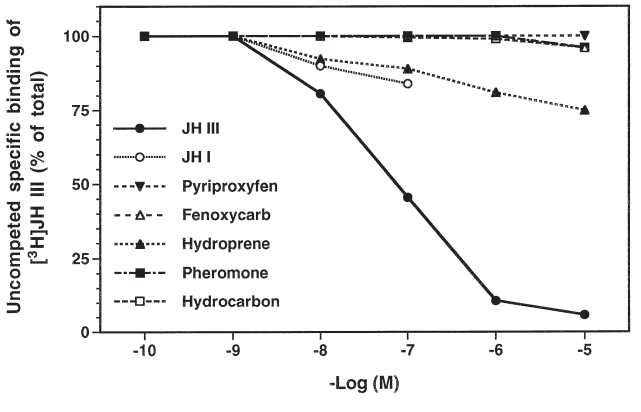

FIGURE 6. Competitive displacement of [^{3}H](10*R*)JH III by racemic JH III and JH I, hydrocarbon, contact sex pheromone, and three JH analogs (pyriproxyfen, fenoxycarb, and *S*-hydroprene). Lipophorin was incubated with 10 nM [^{3}H](10*R*)JH III and increasing amounts of unlabelled ligands. The values are expressed as a percentage of the total [^{3}H](10*R*)JH III bound. The values are the means of three determinations.

The results presented in this paper clearly show that high density lipophorin isolated from the hemolymph of adult females binds the natural (10*R*)JH III. Scatchard analysis indicates the presence of a single high affinity binding site that appears to be saturable and specific to (10*R*)JH III. The apparent dissociation constant, K_d, is 9.75 nM (Fig. 5). The binding characteristics of *B. germanica* lipophorin show many similarities to JH-binding lipophorin from other insects, including the Colorado potato beetle, *L. decemlineata* (de Kort and Koopmanschap, 1987), the fruitfly *D. melanogaster* (Shemshedini and Wilson, 1988), the honey bee, *A. mellifera* (de Kort and Koopmanschap, 1986), and the cockroaches *P. americana* (de Kort and Koopmanschap, 1986), *L. maderae* (Rayne and Koeppe, 1988), *N. cinerea* (Kindle *et al*., 1989; Lanzrein *et al*., 1993) and *D. punctata* (King and Tobe, 1988, 1992). The dissociation constants vary considerably among these insects and range from 1.5 to 157 nM (Trowell, 1992). In cockroaches, however, K_d values are at the lower end of this range, generally below 10 nM. Importantly, it appears that K_d values can depend to some extent on the methods used to derive them. Park *et al*. (1993) concluded that using equilibrium dialysis to separate bound from free JH resulted in at least a 10-fold reduction in K_d values. Lower K_d values also result when competitive binding assays include purified JHBP rather than hemolymph and natural rather than racemic ligands (Prestwich *et al*., 1987). For example, in the cockroach *N. cinerea*, lipophorin bound natural (10*R*)JH III 46-fold more strongly than it bound the (10*S*) enantiomer (Kindle *et al*., 1989).

To gain further insight into the dynamics of JH binding to lipophorin we have determined lipophorin concentration in the hemolymph by enzyme-linked immunosorbent assay (ELISA), and the hemolymph JH titer by gas-liquid chromatography–mass spectrometry. Our preliminary results indicate that the concentration of JH reaches a maximum of 0.06 nmol/mg of lipophorin (unpublished). Because *Blattella* lipophorin has a high affinity JH III binding site and the amount of JH present in the hemolymph is four times lower than the maximum binding capacity (B_{max}), these results suggest that virtually all circulating JH must be bound to lipophorin, even when JH titer peaks in vitellogenic females.

Since the advent of photoaffinity labelling, it has become a valuable tool in characterizing the binding sites of transport proteins and receptors in a wide variety of insects (Prestwich *et al*., 1994). By using [^{3}H]EFDA, a diazoacetate photoaffinity analog of JH III, we have demonstrated that the JH III binding site is associated with apoLp-I (M_r 212 kDa) and, assuming that exposure to UV light did not covalently modify apoLp-II, this smaller subunit appears to lack JH binding sites. ApoLp-I has

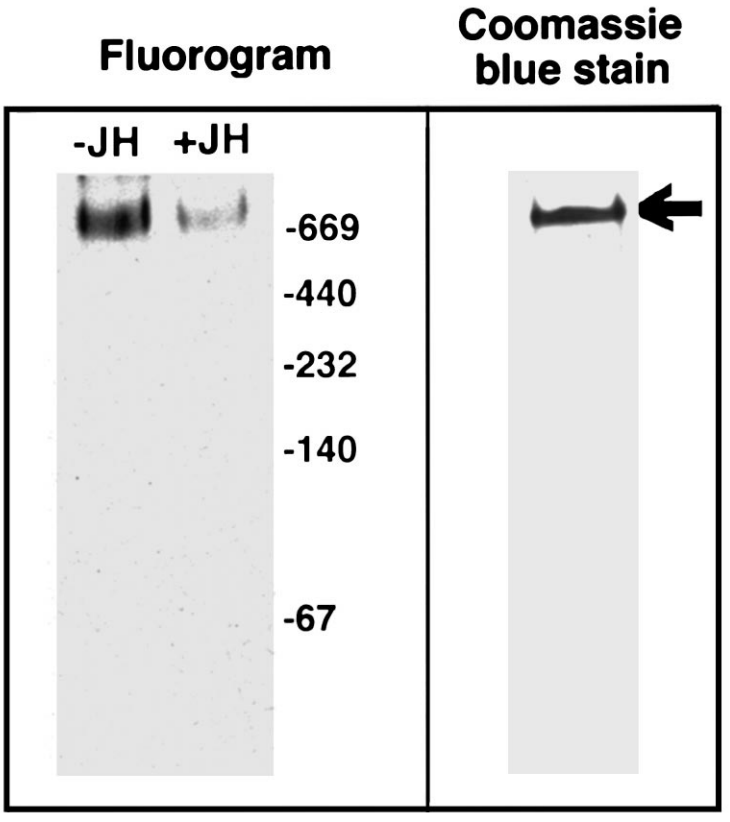

FIGURE 7. Fluorogram of lipophorin labelled with [^{3}H]EFDA (left panel). Lipophorin was incubated with [^{3}H]EFDA in the presence (+ JH) or absence (− JH) of 200-fold excess unlabelled racemic JH III and subjected to native polyacrylamide gel electrophoresis. The right panel shows the Coomassie blue stained native gel for comparison. The arrow indicates the lipophorin band.

also been identified as a JH binding site-containing subunit in the cockroaches *L. madareae* (Koeppe *et al*., 1984; Rayne and Koeppe, 1988), *D. punctata* (King and Tobe, 1992), and tentatively in *P. americana* (de Kort and Koopmanschap, 1989), as well as in the termite *R. flavipes* (Okot-Kotber and Prestwich, 1991).

Juvenile hormone binding proteins are JH homolog-selective (Prestwich *et al*., 1987). Lipophorin exhibits maximal binding to JH III and lower affinities for higher JH homologs. For example, in the American cockroach, lipophorin bound JH III with higher affinity than it did JH I (de Kort and Koopmanschap, 1989). We now extend these findings to the German cockroach. Moreover, we have used three JH analogs to test whether they compete for JH binding sites. The data show that hydroprene, which has a similar sesquiterpenoid backbone to that of JH III, is somewhat effective at displacing the natural (10*R*)JH III, but only at high concentrations. Other JH analogs that are structurally distinct from JH III are unable to compete for JH III binding sites. This finding is intriguing because fenoxycarb and pyriproxyfen have been shown to have potent JH-like effects on *Blattella* and are indeed effective at lower concentrations than hydroprene (Bennett and Reid, 1995). If the JH analogs are also shuttled through the hemolymph by lipophorin, these data suggest that lipophorin might contain distinct binding sites for the natural hormone and for JH analogs. This hypothesis is attractive because a similar phenomenon was observed with the nuclear binding sites in *Manduca* (Osir and Riddiford, 1988). However, studies with *D. melanogaster* suggest that excess unlabelled JH III or JH analogs do not compete for hemolymph JH-analog binding sites; JH analogs might thus bind to non-specific, high capacity binding sites (Shemshedini and Wilson, 1988). Likewise, excess JH III or JH analog (methoprene) failed to displace competitively a radiolabelled photoaffinity analog of methoprene from apoLp-I (Okot-Kotber and Prestwich, 1991).

Lipophorin is a multi-functional vehicle for the transport and distribution of both neutral and polar lipids among various tissues (for review see Chino, 1985; Kanost *et al*., 1990; Law *et al*., 1992; Van der Horst *et al*., 1993; Blacklock and Ryan, 1994; Soulages and Wells, 1994). In addition, lipophorin has been shown to function as a plasma coagulogen in the cockroach *L. maderae* (Barwig, 1985), and participates in detoxification and immunological responses in *Bombyx mori* (Kato *et al*., 1994). Recent work in our laboratory showed that in *B. germanica*, lipophorin functions as a carrier for contact pheromone and cuticular hydrocarbon (Gu *et al*., 1995) and for phospholipid and diacylglycerol (unpublished). The present study adds another function for lipophorin in *B. germanica*, as a JH carrier. Moreover, our competitive binding studies indicate that phero-

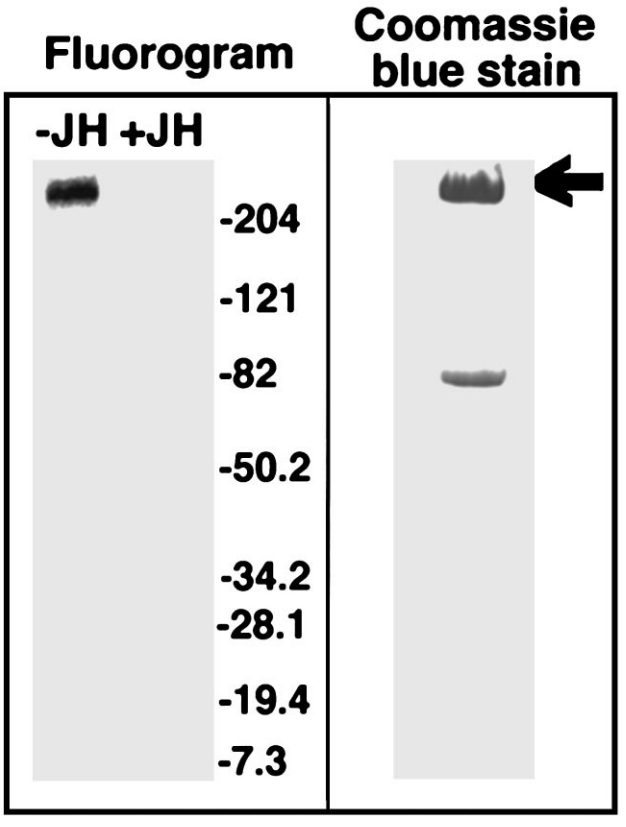

FIGURE 8. Fluorogram of lipophorin labelled with [^{3}H]EFDA (left panel). Lipophorin was incubated with [^{3}H]EFDA in the presence (+ JH) or absence of (− JH) 200-fold excess unlabelled racemic JH III and subjected to SDS–PAGE. Coomassie blue-stained SDS–PAGE gel is shown in the right panel. The arrow indicates apoLp-I.

mone and hydrocarbon do not compete for the JH III binding site (Fig. 6). Although it is possible that distinct, high affinity binding sites are available on lipophorin for hydrocarbon and pheromone, structural data support an alternative model. Like mammalian lipoproteins, lipophorin contains a core of non-polar lipids surrounded by an outer shell of phospholipids and proteins (Soulages and Wells, 1994). In *B. germanica* large amounts of hydrocarbons associate with lipophorin (Gu *et al.*, 1995), and they are presumably located in its inner core. This, together with the lack of species-specific binding of hydrocarbons to lipophorin (Chino, 1985), suggests that hydrocarbons might partition into lipophorin non-specifically. Multiple functions and transport of various lipid ligands also suggest that lipophorin titers might not be easily related to changing titers of any one of its ligands. Our studies are directed at understanding the functions of lipophorin and the molecular mechanisms of ligand-specific interactions with lipophorin binding sites and with target tissues.

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Acknowledgements—This research was supported in part by the Blanton J. Whitmire Endowment at North Carolina State University and by grants from the USDA-NRI (9501922) and NSF (IBN-9407372) to C. Schal. We thank the following for gifts of chemicals: Dr R. Nishida (pheromone), Dr G. D. Prestwich (radiolabelled EFDA), Dr R. M. Roe (OTFP), Dr Maag (fenoxycarb), Zeneca (pyriproxyfen), and Sandoz (hydroprene). We also thank Drs P. Neese and R. M. Roe for use of the radio-HPLC and E. Armstrong and L. Castrillo for technical assistance. P. Neese, H. Young and two anonymous reviewers provided critical comments on the manuscript.