





# Isolation and Fluorescence Studies on a Lipophorin From the Weevil *Diaprepes abbreviatus*

Jeffrey P. Shapiro

U.S. Horticultural Research Lab, Agricultural Research Service, U.S. Department of Agriculture, Orlando, Florida

Lipophorin was isolated from larvae of a root weevil, *Diaprepes abbreviatus* (Coleoptera: Curculionidae), using density gradient ultracentrifugation. *D. abbreviatus* lipophorin contained two apoproteins, apolipophorin-I ( $M_r = 226,000$ ) and apolipophorin-II ( $M_r = 72,100$ ) and had a density of 1.08. Relative to other larval lipophorins, *D. abbreviatus* lipophorin contained little cysteine (determined as cysteic acid) and methionine. Fluorescence spectroscopy of intrinsic tyrosine and tryptophan residues excited at 290 nm revealed a single broad emission peak at 330 nm. Upon denaturing and delipidating lipophorin in guanidine HCl, this peak resolved into two peaks with maxima at 305 and 350 nm. Excitation spectra suggested that the two peaks were due to tyrosine and tryptophan, respectively. Fluorescence quenching agents, iodide and acrylamide, were used to determine accessibility of tyrosine and tryptophan residues to the aqueous environment. Iodide, a polar quenching agent, did not quench fluorescent emission from native lipophorin; quenching by iodide increased to moderate levels when lipophorin was denatured in guanidine HCl. Acrylamide quenched the fluorescence of native lipophorin moderately and very efficiently quenched fluorescence of denatured lipophorin. No difference was observed between fluorescence quenching of denatured vs. denatured and delipidated lipophorin by either iodide or acrylamide.

**Key words:** insect lipoprotein, spectrofluorometry, protein chemistry, fluorescence quenching, Coleoptera, Curculionidae

**Acknowledgments:** I thank Bongwoo Roe and Denise Dunn for excellent technical assistance, Dr. Richard T. Mayer for providing expert technical consultation regarding fluorometric methods, and Dr. John K. Kawooya for providing the method for extraction of lipids from lipophorin in guanidine HCl. Thanks also to Dr. William J. Schroeder, Schuyler Lovstrand, and Rouselle Sutton for providing larval *D. abbreviatus* throughout this study, and to Dr. John Law and Wallace Clark for amino acid analyses.

Mention of a company name or proprietary product does not constitute an endorsement by the U.S. Department of Agriculture.

Received April 21, 1987; accepted December 17, 1987.

Address reprint requests to Dr. J.P. Shapiro, USDA, ARS, 2120 Camden Road, Orlando, FL 32803.

## INTRODUCTION

In every order and stage of insect studied to date, lipophorins constitute a major portion of total hemolymph protein, and in most stages are the only lipoprotein present. Among insect orders, the lipophorins exhibit common characteristics in molecular weights of their apoproteins, high contents of phospholipid and diacylglycerol [1, 2], and partial immunologic identity of apoLp-II\* [3]. However, physiologic functions and the lipid-to-protein ratios (and thus densities) vary considerably, indicating great diversity in metabolic and adaptive function of lipophorins [4].

One demonstrated capacity of lipophorin and another major hemolymph protein, arylphorin, is to bind apolar or semipolar xenobiotics. Insecticides have been shown to bind *in vivo* to lipophorin [5] and *in vitro* to lipophorin and arylphorin [6] from lepidopteran species. As yet, there is no evidence that hemolymph proteins enhance either toxication or detoxication by binding xenobiotics. Hemolymph proteins may facilitate toxication by either increasing absorptive rates or distributing the toxins directly to target organs; they may facilitate detoxication either by acting as a sink to sequester toxicants or by transporting toxicants to metabolic and excretory sites. However, little is known of mechanisms of insecticide uptake and distribution [7], especially through oral routes of introduction, and even less is known of the specific roles of hemolymph proteins in toxication and detoxication.

We are using the root weevil *Diaprepes abbreviatus* to explore the roles of hemolymph proteins in uptake and distribution of toxins. *D. abbreviatus* was introduced into Florida from the Caribbean basin in about 1964 [8] and has since become an economic pest of Florida citrus. Larval *D. abbreviatus* destroy citrus plants by attacking the cortex of the primary root system, and are difficult to detect and control, due to their subterranean habitat. *D. abbreviatus* therefore may be a good candidate for control by the selective breeding or genetic engineering of citrus rootstocks for resistance. Development of resistant plants might be expedited through knowledge of the biochemical basis of insect toxication by plant products.

We have therefore isolated and partially characterized the lipophorin from *D. abbreviatus* in an effort to understand the role of hemolymph proteins in absorption and disposition of natural xenobiotics. Since hydrophobic characteristics of lipophorin and arylphorin will be important in binding apolar xenobiotics, we probed the intrinsic fluorescence of apolar tyrosine and tryptophan residues in lipophorin. These residues proved nearly inaccessible to the hydrophilic fluorescence quencher iodide, even in dissociated and delipidated lipophorin in guanidine HCl.

\*Abbreviations: apoLp-I, -II = apolipoproteins-I and -II; DFP = diisopropylfluorophosphate; FITC-Con A = fluorescein isothiocyanate-derivatized concanavalin A; HDLp = high-density lipophorin; isothiocyanate-concanavalin A; MESA = mercaptoethanesulfonic acid; PAGE = polyacrylamide gel electrophoresis; PBS = phosphate-buffered saline (0.15 M NaCl/0.10 M sodium phosphate/0.05% EDTA/0.05% sodium azide); PTU = phenylthiourea; SDS = sodium dodecyl sulfate.

## MATERIALS AND METHODS

### Insects

Lipophorin was isolated from last-instar larvae weighing 300–900 mg each. Larvae were reared on an artificial diet [9] from eggs that were obtained from a laboratory colony established from adults collected in the field in 1985 [10].

### Lipophorin Isolation

The vertical rotor method developed for isolation of *Manduca* lipophorin [11] was modified for the SW56Ti (now the SW 60) swinging bucket rotor (Beckman, Palo Alto, CA). Hemolymph (50–100  $\mu$ l/larva) was collected from larvae using 100- $\mu$ l glass capillary pipets. Upon collection, hemolymph was pooled in a 1.5-ml microcentrifuge tube held on ice. The microcentrifuge tube contained 5  $\mu$ M DFP and approximately 1 mg crystalline PTU (Sigma, St. Louis, MO) in 200  $\mu$ l of PBS.

Pooled hemolymph from 20–40 larvae (< 1.5 ml total volume) was added to 1.11 g KBr, diluted to 2.5 ml with PBS, mixed, and centrifuged for 3 min at 1,000g and 4°C in a refrigerated centrifuge (IEC Centra 7R, Needham Heights, MA). The supernatant was added to a 4.4 ml nitrocellulose ultracentrifuge tube and overlaid with an equal volume of 0.9% NaCl. After centrifuging for 16 h at 46,500 rpm (206,000g) at 10°C, the clear supernatant was withdrawn and the yellow lipophorin band collected through the top of the tube with a Pasteur pipet.

For density gradient analysis, fractions were collected from the top of the tube with a Pipetman (Rainin Instr. Co., Woburn, MA). Absorbances of fractions from tubes that contained lipophorin were read spectrophotometrically with an LKB (Gaithersburg, MD) Ultrospec 4050 at a wavelength of 280 nm. Densities of fractions from blank tubes that contained KBr, PBS, and NaCl (above) but lacked hemolymph were determined gravimetrically. Tubes that contained only KBr were ultracentrifuged with tubes that contained hemolymph dialyzed against KBr solution (see Results). Refractivities were read on an AO/Reichert (Buffalo, NY) Abbe Mark II refractometer, and densities were calculated by interpolation from KBr refractivity tables [12].

### Protein Determinations

Protein determinations were made routinely either by the Folin phenol method of Peterson [13], employing SDS for efficient protein solubilization, or by absorbance at 280 nm in native form. The ratio of absorbance at 280 nm to protein concentration in mg/ml by the Folin phenol method, using a gamma globulin standard (BioRad, Richmond, CA), was 1.08.

Gravimetric protein determinations were made on an AE163 balance (Mettler Instrument Corp., Hightstown, NJ; readability to 10  $\mu$ g) after carefully measuring the absorbance at 280 nm of lipophorin in 25% PBS. Polyethylene microcentrifuge tubes were tared after heating at least 1 h at 110°C and cooling to room temperature in an evacuated desiccator. A quantity of lipophorin equivalent to 1.0 ml at an optical density of 1.0 at 280 nm was either dialyzed extensively against distilled water or delipidated by a modification of the procedure described by Osborne [14]. Each aliquot of dialyzed lipo-

phorin was quantitatively transferred to a tube, heated 2 h at 110°C, desiccated in the heating block for 2 h, and cooled in the desiccator to room temperature for 2 h prior to weighing.

For delipidation, lipophorin (125 or 205  $\mu\text{l}$ ), dialyzed extensively against 25% PBS, was slowly added to a tube containing 300  $\mu\text{l}$  of methanol while mixing on a vortex mixer. Ether (700  $\mu\text{l}$ ) was then added, thoroughly mixed, and allowed to stand on ice 10 min. Protein was pelleted on a microcentrifuge (Beckman model 11) for 4 min at 10,000 rpm. The supernatant was discarded, 1 ml of diethyl ether was added, mixed, recentrifuged as above, and the ether was removed. Distilled water (500  $\mu\text{l}$ ) was then added and mixed with the protein pellet, centrifuged, and water was removed. This was repeated, and tubes were dried and weighed as above.

### Electrophoresis

Twenty to forty microliters of sample, boiled 5 min in buffer that contained SDS and  $\beta$ -mercaptoethanol, were applied to 15  $\times$  20 cm  $\times$  1.5 mm, 4-15% acrylamide gradient slab gels [15]. High- and low- $M_r$  protein standards were from BioRad (Richmond, CA). Gels were run at 15°C and 15 mA constant current per gel, stained with Coomassie brilliant blue 2R (BioRad) in 50% methanol/10% acetic acid, and destained first in 50% methanol/10% acetic acid and then in 5% methanol/10% acetic acid.

For apoprotein molecular weight estimates, five lanes each of lipophorin (1  $\mu\text{g}/\text{lane}$  in 20  $\mu\text{l}$  of sample buffer) and standards (0.25  $\mu\text{l}/\text{lane}$ ) were run on each of three individual slab gels as above. The distance of each band from the top of the separating gel to the center of the band was measured, distances were averaged, linear regression of  $\log M_r$  vs. distance migrated was calculated, and  $M_r$  of the apolipophorins was estimated.

High-mannose carbohydrates were detected by incubation of FITC-Con A (Sigma Chemical Co., St. Louis, MO) with fixed, unstained SDS-PAGE gels [16].

### Spectrofluorometry and Spectrophotometry

Spectrofluorometry was done on an SLM/Aminco SPF-500C spectrofluorometer (Urbana, IL) interfaced with an IBM personal computer. Spectra were taken from samples in 1-cm pathlength cuvettes and were baseline-corrected by subtracting spectra run in the sample buffers less protein.

Lipophorin was prepared for fluorescence quenching studies by denaturation in guanidine HCl (UV grade; United States Biochemical Corp., Cleveland, OH), or denaturation followed by delipidation. Lipophorin (11 mg/ml) in KBr was denatured by diluting to 400  $\mu\text{g}/\text{ml}$  in 6 M guanidine HCl/20 mM Tris/0.2 M NaCl/pH 8.4, heating to 100°C for 20 min, and diluting to 100  $\mu\text{g}/\text{ml}$ . Delipidated lipophorin was prepared by denaturing in guanidine HCl as above, mixing with ethanol, and partitioning against ether (J. Kawooya, personal communication). For studies on native lipophorin, an 11-mg/ml solution in KBr was diluted to 100  $\mu\text{g}/\text{ml}$  in 2 ml of PBS.

Fluorescence quenching curves were obtained by titrating 100  $\mu\text{g}/\text{ml}$  lipophorin preparations with 3 M KI reduced with 1 or 2 mM sodium thiosulfate

(Fisher Chem. Co., Orlando, FL) or with freshly prepared 3 M acrylamide (electrophoresis grade, 99.9% pure; BioRad, Richmond, CA). Excitation at 290 nm minimized inner filter effects of acrylamide and iodide. An excitation wavelength of 290 nm and an emission wavelength of 350 nm excluded most of the tyrosine emission.

### Amino Acid Analysis

Prior to analysis, lipophorin (500–1200 pmol) was delipidated with methanol and ether in polyethylene microcentrifuge tubes, using the procedure of Osborne [14], except that volumes used were reduced to 10% of those given.

Amino acid determinations were made at the University of Arizona, Department of Biochemistry (Tucson, AZ), in a Beckman 7300AAA amino acid analyzer with a 20-cm sodium high-performance ion exchange column. For determination of all amino acids except Cys, samples were hydrolyzed for 24 h in 3 N MESA (Pierce, Rockford, IL) at 110°C. Analyses were confirmed with duplicate samples hydrolyzed in 0.5 ml of 6 N constant boiling HCl (Pierce) in Pierce Vacutubes. Prior to analysis, samples from MESA hydrolysis were diluted 11-fold in Beckman sample buffer NA-S, while HCl hydrolyzates were lyophilized and resuspended in the sample buffer.

Cysteic acid was determined after oxidizing in performic acid [17], lyophilizing, and hydrolyzing 24 h with 6N HCl as above. After determining the Tyr/Trp ratio using second-derivative spectrophotometry [18] on a DW2000 spectrophotometer (SLM/Aminco, Urbana, IL) equipped with an IBM PC-XT computer, Trp content was calculated from the Tyr content, determined as above. Trp content was corroborated by amino acid analysis following MESA hydrolysis.

## RESULTS

### Isolation

Isolation of lipophorin from 1 or 2 ml of *D. abbreviatus* hemolymph, using a low-volume VTi65 vertical rotor, yielded a slightly heterogeneous preparation. The procedure was therefore adapted to an SW56Ti swinging bucket rotor, yielding purer preparations after centrifuging 16 h at 206,000g. The resulting gradient (Fig. 1A) designed to give a midtube density identical to that of the VTi50 rotor (1.15 g/ml), was broader than that formed in the VTi50 rotor (1.00–1.33 g/ml in the SW56Ti vs. 1.03–1.28 g/ml in the VTi50 [11]). Since 24 h of centrifugation produced a density gradient identical to one produced after 16 h (results not shown), gradients were judged to be at equilibrium after 16 h.

Isolated larval *D. abbreviatus* lipophorin was homogeneous on SDS-PAGE, i.e., the only proteins that it contained were apoLp-I and apoLp-II (Fig. 2, fraction 4; equivalent to fraction 5 in Fig. 1A, due to differences in the volume/fraction). Lipophorin floated at a KBr density of 1.08 g/ml. Isolated lipophorin was recentrifuged in the same system after dialysis against pure 44.3% KBr in water, and densities were measured by refractivity to assure that PBS and NaCl used in the routine gradient did not give erroneous

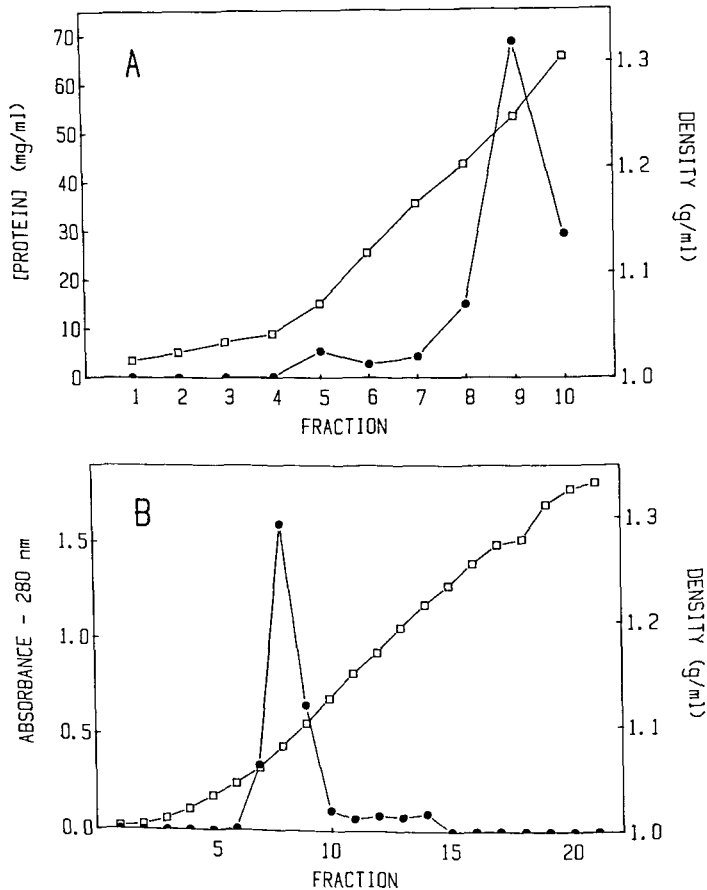


Fig. 1. Preparative density gradient ultracentrifugation and density determination upon recentrifugation. (A) Preparation: Hemolymph was collected and centrifuged as described, and fractions of 440  $\mu$ l collected from the centrifuge tube. Densities ( $\square$ — $\square$ ) were determined gravimetrically and protein concentrations ( $\bullet$ — $\bullet$ ) were determined by protein assay [13]. (B) Density determination: Two hundred microliters of lipophorin (4.5 mg/ml) were removed from a density gradient and dialyzed for 1.5 days against 4 changes of 500 ml 44.3% KBr in water. The lipophorin was then diluted to 2.5 ml with 44.3% KBr, overlaid with water in a centrifuge tube, and ultracentrifuged opposite a tube that contained KBr dialyzate overlaid with water (for density determination via refractometry). Fractions (235  $\mu$ l) were collected from top to bottom of both tubes. Densities ( $\square$ — $\square$ ) were determined by KBr refractivity and absorbance ( $\bullet$ — $\bullet$ ) was read at 280 nm.

results due to deviation from KBr refractivity. Density of the recentrifuged lipophorin and profile of the density gradient (Fig. 1B) were identical to those of the standard gradient.

#### Amino Acid Composition and Specific Absorbance of Lipophorin

Amino acid analysis revealed a composition similar to other lipophorins (Table 1). The ratio of tyrosine to tryptophan residues in lipophorin was 7.9, as determined by second derivative spectroscopy.



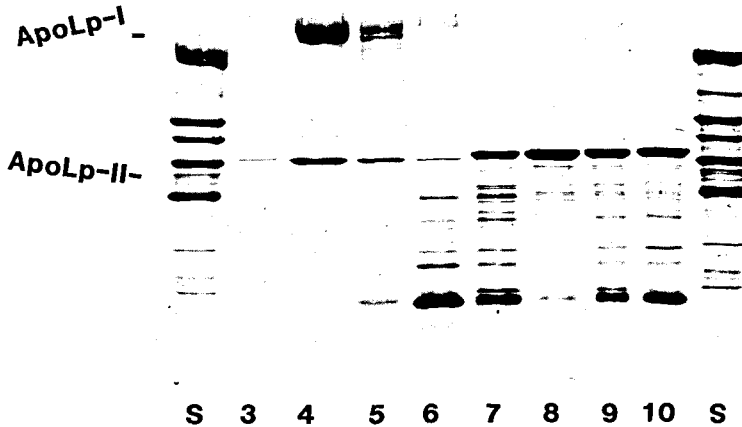


Fig. 2. SDS-PAGE of density gradient fractions. Larval hemolymph was routinely centrifuged as in Figure 1A, except that fractions were  $470 \mu\text{l}$  each. Fractions were microdialyzed overnight against PBS in a BRL apparatus (Bethesda, MD) ( $M_r$  cutoff = 6-8,000), and applied to a  $15 \text{ cm} \times 20 \text{ cm} \times 1.5 \text{ mm}$  4-15% acrylamide gradient slab gel, Coomassie stained as described. ApoLp-I and apoLp-II indicate apoproteins appearing in lanes 4 and 5. Standards (S) are described in Figure 3 legend.

By gravimetric determination, 1.0 ml of lipophorin at an optical density of 1.0 at 280 nm had a total dry mass of  $1.65 \pm 0.12 \text{ mg}$  ( $n = 5$ ) and a delipidated dry mass of  $0.74 \pm 0.14 \text{ mg}$  ( $n = 7$ ; means  $\pm$  SD). Lipid was thus 55% of the total mass.

#### Apoprotein Molecular Weights and Carbohydrate Content

The two or three component proteins of lipophorins have been termed apolipophorins [11], in accord with standard terminology defining apolipoproteins as the component proteins of a lipoprotein removed from their characteristic prosthetic groups, i.e., lipids. Molecular weights of the two larval apolipophorins were estimated from a linear regression of  $\log M_r$  vs. mobility of standards on two separate gels. Five replicates each of standards and lipophorin were run on each gel. Gels were loaded with the minimal quantity of lipophorin required for clear Coomassie staining, yielding very thin bands and greater accuracy of measurement (Fig. 3). The two gels yielded an apoLp-I molecular weight of 226,000 and an apoLp-II molecular weight of 72,100. Standard deviations of apolipophorin mobilities within a gel ( $n = 5$ ) were less than 1.6%.

Both apoproteins contained quantities of mannose detectable by incubation of fixed, unstained SDS-PAGE gels with FITC-con A [16] (results not shown).

TABLE 1. Amino Acid Composition of Delipidated Lipophorin

Amino Acid	Residues per mole <sup>1</sup>	Mole percent <sup>2</sup>
Asp	307	13.8
Thr	110	4.9
Ser	162	7.3
Glu	259	11.7
Pro	93	4.2
Gly	163	7.3
Ala	175	7.9
Cys/2	7	0.3
Val	132	5.9
Met	7	0.3
Ile	131	5.9
Leu	232	10.4
Tyr	71	3.2
Phe	89	4.0
His	32	1.4
Lys	200	9.0
Arg	61	2.8
Trp	9	0.4
Total	2238	100.0

<sup>1</sup>Based on the following molecular weights:

ApoLp-I = 226,000

ApoLp-II = 72,100

Total protein = 298,100

<sup>2</sup>Mean of duplicate determinations

### Fluorescence Spectra

The fluorescence spectra of lipophorin in native, dissociated, and delipidated states were examined (Fig. 4). Native lipophorin in PBS, excited at 280 nm, revealed one broad emission peak between 300 nm and 400 nm, with a maximum at 330 nm (Fig. 4A). The emission spectrum obtained from lipophorin in 44.4% KBr was identical to the spectrum in PBS.

When lipophorin was denatured and dissociated in 6 M guanidine HCl, the broad peak seen in native lipophorin resolved into a shoulder at 310 nm and a peak at 350 nm. (Dissociation of the two apoproteins has been demonstrated by their independent isolation on a guanidine HCl-packed Sepharose CL6B column; Shapiro, unreported data.) When the denatured lipophorin was also delipidated by partitioning in guanidine HCl-ethanol against diethyl ether (method of J. Kawooya, personal communication), a prominent emission peak replaced the shoulder at 305 nm (Fig. 4A).

The two emission peaks—at 305 nm and 350 nm—were identified as primarily due to tyrosine and tryptophan, respectively, by comparing excitation spectra collected at emission wavelengths of 305 nm or 350 nm (Fig. 4B). Excitation maxima corresponded closely to the absorption maxima reported for the two amino acids in solution [19].

### Quenching of Intrinsic Fluorescence

Accessibility of tryptophan residues to an ionic and an apolar quenching agent was investigated. Intact guanidine HCl-treated or delipidated lipo-

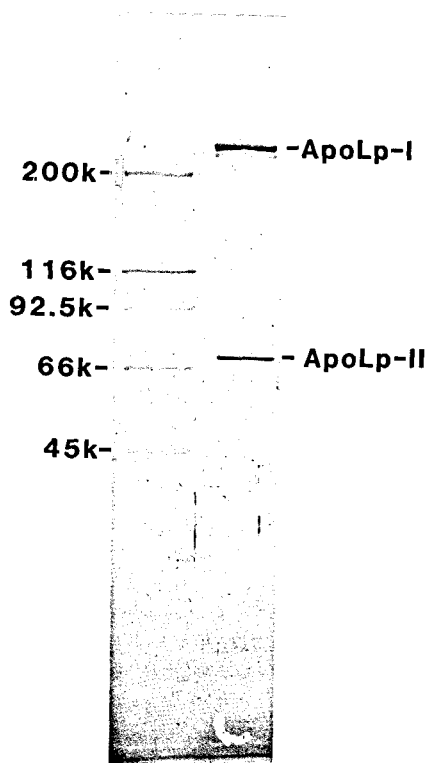


Fig. 3. SDS-PAGE for use in molecular weight determination. Two lanes of standards and lipophorin apoproteins are shown. Proteins were applied as in Materials and Methods. Protein standards (S): myosin ( $M_r = 200,000$ ),  $\beta$ -galactosidase ( $M_r = 116,000$ ), phosphorylase B ( $M_r = 92,500$ ), bovine serum albumin ( $M_r = 66,200$ ), ovalbumin ( $M_r = 45,000$ ).

phorin was titrated with increasing concentrations of either acrylamide, which can penetrate hydrophobic protein matrices, or iodide ion, which cannot [20].

Iodide proved to be an ineffective fluorescence quencher. Even when lipophorin was dissociated in guanidine HCl, there was little fluorescence quenching, though some increase in the quench ratio ( $F_0/F$ , ratio of fluorescence intensity without quencher to intensity with quencher) did occur (Fig. 5A).

In contrast, acrylamide effectively quenched the fluorescence of denatured and delipidated lipophorin, as indicated by a relatively large increase in quench ratio with increasing acrylamide concentration (Fig. 5B).

## DISCUSSION

The isolation and fluorometric characterization of lipophorin from *D. abbreviatus* are the bases for our investigations on its role in transport of synthetic and plant-derived xenobiotics. The modified procedure for isolation of *D. abbreviatus* lipophorin makes use of low volumes of hemolymph (0.5 ml

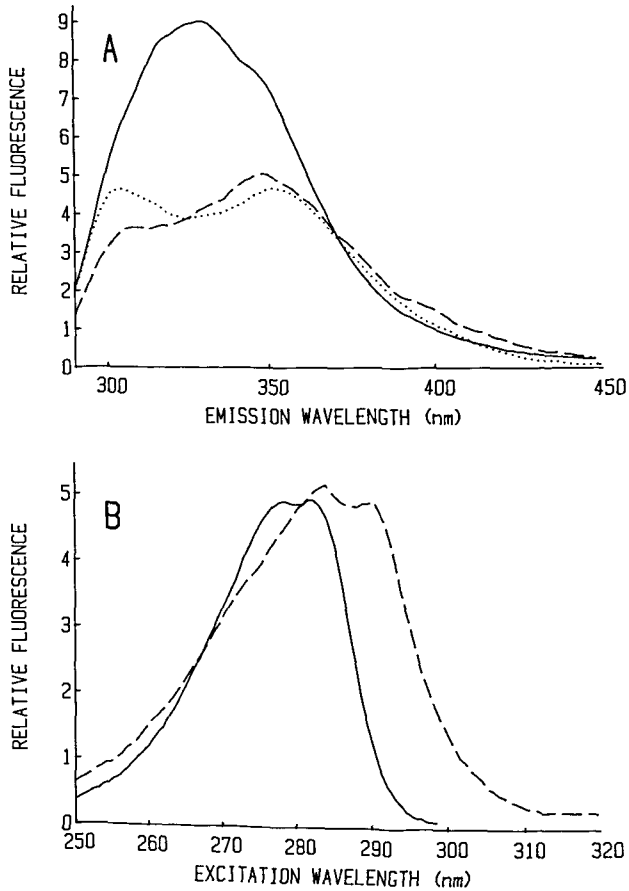


Fig. 4. Fluorescence spectra of intact and denatured lipophorin. A) Emission spectra of native lipophorin (—), lipophorin denatured in guanidine HCl (---), or denatured and delipidated (····). Excitation was held constant at 280 nm, 25°C. B) Excitation spectra of guanidine HCl-treated and delipidated lipophorin, with emission wavelength held constant at 305 nm (—) or 350 nm (---).

to 10 ml). The HDLp isolated from larvae by this procedure had a density of 1.08 g/ml, lower than that exhibited by *Manduca sexta* (1.15 g/ml) [11], honey bee (1.13 g/ml) [21], or southwestern corn borer (1.11 g/ml) [22] larval HDLp. Molecular weights of *D. abbreviatus* apoproteins also were significantly lower than those of any of the above larval lipophorins.

The amino acid composition of larval *D. abbreviatus* was similar to the compositions of other lipophorins. *D. abbreviatus* was low in cysteine and methionine content, however. These two residues were only 20–50% as abundant as in larval lipophorins of other insects [11,21,22].

Spectrofluorometry has not been previously applied to the characterization of lipophorin, and offers new insight into environments of the aromatic amino acids tyrosine and tryptophan. The fluorescence emission spectra revealed changes in tyrosine and tryptophan fluorescence as apoproteins

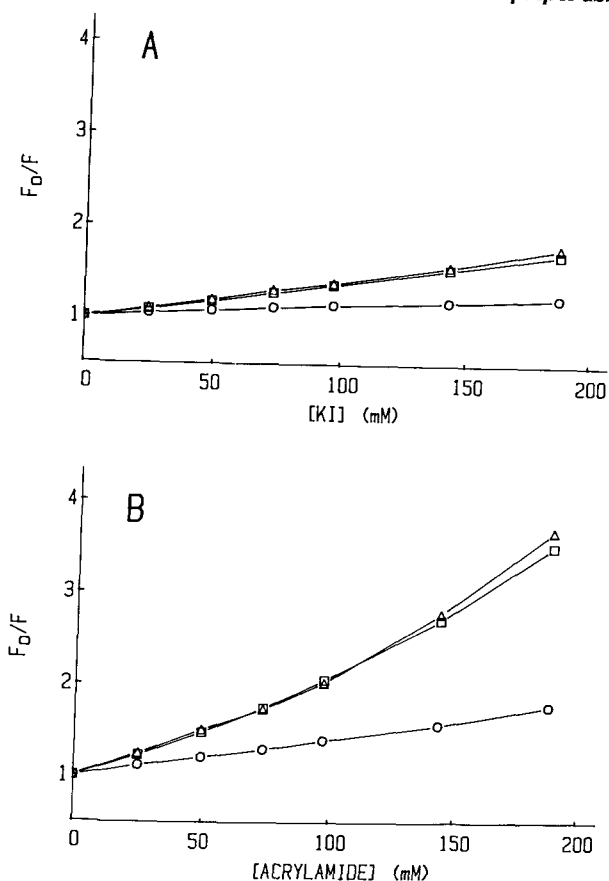


Fig. 5. Fluorescence quenching curves of lipophorin in native form (○—○), denatured in guanidine HCl (□—□) or denatured and delipidated (△—△), quenched by (A) KI or (B) acrylamide. Lipophorin was treated as above. Emission and excitation wavelengths were 350 nm and 290 nm, respectively.

were denatured and dissociated from each other, and as lipids were stripped from the apoproteins.

A broad peak that was revealed through excitation of native lipophorin at 280 nm resolved into two discrete peaks upon denaturation and delipidation (Fig. 4A), in a manner similar to that of an insect fatty acid synthase denatured in SDS [23]. As in the present study, addition of either SDS or sodium cholate to the synthase lipid-protein complex resulted in a bathochromic shift of emission, indicating increased polarity of tryptophan environments. With addition of SDS to the synthase, an emission peak at 305 nm also appeared.

As indicated by the lipophorin excitation spectra, the peaks of emission at 305 and 350 nm are probably due to fluorescence of tyrosine and tryptophan residues, respectively, although tyrosinate can mimic the tryptophan spectrum under some conditions [19]. The resolution into two peaks of emission seems due to decreased emission at intermediate wavelengths around 330 nm, with a decrease in total quantum yield accompanying denaturation. It

therefore seems that the emission of one population of tryptophan residues exhibits a bathochromic wavelength shift upon denaturation, from approximately 330 nm to 350 nm. This red shift typically reflects increased polarity of tryptophan microenvironments [19].

Denaturation and delipidation are responsible for further increases in tyrosine emission at 305 nm (Fig. 4A). This is undoubtedly due to increasing intramolecular distances between tyrosine and tryptophan, resulting in decreased energy transfer from the former to the latter. Proximity of tyrosine to tryptophan seems to be maintained by residual lipids, even after dissociation in guanidine HCl. In *M. sexta*, lipids remain bound to apoLp-I, but not apoLp-II after dissociation by treatment with guanidine HCl (J. Kawooya, personal communication). The binding of lipids in the presence of guanidine HCl is supported by spectrofluorometric studies on apoproteins isolated by Sepharose CL-6B gel permeation chromatography in guanidine HCl (Shapiro, unreported data).

Fluorescence quenching studies demonstrated a relative ease of quenching of lipophorin by acrylamide, in contrast to quenching by iodide (Fig. 5). Denaturation by guanidine HCl increased the availability of tryptophan to the aqueous environment, and therefore to both acrylamide and iodide. However, although iodide was a slightly more effective quencher when lipophorin was denatured, iodide still did not quench to the same extent as acrylamide.

Tryptophan-quenching studies may indicate the existence of several intrinsic fluorophores or classes of fluorophore, in relation to interactions with the quencher. Acrylamide fluorescence quenching curves (Fig. 5B) demonstrate upward curvature. This may result from excitation of several classes of quenched tryptophan, e.g., of statically vs. dynamically quenched residues [20].

The characterization of some basic fluorometric characteristics of a lipophorin will contribute to ongoing studies of structure and function. Fluorometry utilizing intrinsic and extrinsic probes may effectively highlight structural features, permit studies on association of both xenobiotic and natural compounds with the lipophorin particle, and perhaps even elucidate the characteristics of events at the lipoprotein-membrane interface during exchange of lipid.

#### LITERATURE CITED

1. Beenackers AdMTh, Van der Horst DJ, Van Marrewijk WJA: Insect lipids and lipoproteins, and their role in physiological processes. *Prog Lipid Res* 24, 19 (1985).
2. Chino H: Lipid transport: Biochemistry of hemolymph lipophorin. In: *Comprehensive Insect Physiology Biochemistry and Pharmacology*. Kerkut GA, Gilbert LI, eds. Pergamon Press, Vol. 10, pp. 115-135 (1985).
3. Ryan RO, Schmidt JO, Law JH: Chemical and immunological properties of lipophorins from seven insect orders. *Arch Insect Biochem Physiol* 1, 375 (1984).
4. Shapiro JP, Law JH, Wells MA: Lipid transport in insects. *Annu Rev Entomol* 33, 297 (1988).
5. Kawooya J, Keim PS, Law JH, Riley CT, Ryan RO, Shapiro JP: Why are green caterpillars green? *A C S Symp Series* 276, 511 (1985).

6. Haunerland NH, Bowers WS: Binding of insecticides to lipophorin and arylphorin, two hemolymph proteins of *Heliothis zea*. Arch Insect Biochem Physiol 3, 87 (1986).
7. Gilby AR: Cuticle and Insecticides. In: Biology of the Integument. Springer-Verlag, Berlin, pp. 694-702 (1984).
8. Woodruff RE: Citrus weevils in Florida and the West Indies: Preliminary report on systematics, biology, and distribution (Coleoptera: Curculionidae). Fla Entomol 68, 370 (1985).
9. Beavers JB: Biology of *Diaprepes abbreviatus* (Coleoptera; Curculionidae) reared on an artificial diet. Fla Entomol 65, 264 (1982).
10. Schroeder WJ: Induced pupation in *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Fla Entomol 70, 186 (1987).
11. Shapiro JP, Keim PS, Law JH: Structural studies on lipophorin, an insect lipoprotein. J Biol Chem 259, 3680 (1984).
12. Wheast RC: Handbook of Chemistry and Physics. Chemical Rubber Company, Cleveland, Ohio, p. D-192. (1969).
13. Peterson GL: Determination of total protein. Meth Enzymol 91, 95 (1983).
14. Osborne JC Jr: Delipidation of plasma lipoproteins. Meth Enzymol 128, 213 (1986).
15. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T. Nature 227, 680 (1970).
16. Furlan M, Perret BA, Beck EA: Staining of glycoproteins in polyacrylamide and agarose gels with fluorescent lectins. Anal Biochem 96, 208 (1979).
17. Hirs CHW: Determination of cysteine as cysteic acid. Meth Enzymol 11, 59 (1967).
18. Servillo L, Colonna G, Balestrieri C, Ragone R, Irace G: Simultaneous determination of tyrosine and tryptophan residues in proteins by second-derivative spectroscopy. Anal Biochem 126, 251 (1982).
19. Lackowicz JR: Protein fluorescence. In: Principles of Fluorescence Spectroscopy. Plenum Press, New York, pp. 341-381 (1983).
20. Lackowicz JR: Quenching of fluorescence. In: Principles of Fluorescence Spectroscopy. Plenum Press, New York pp. 257-301 (1983).
21. Robbs SL, Ryan RO, Schmidt JO, Keim PS, Law JH: Lipophorin of the larval honeybee, *Apis mellifera* L. J Lipid Res 26, 241 (1985).
22. Dillwith JW, Lenz CJ, Chippendale GM: Isolation and characterization of lipophorin from the hemolymph of diapausing larvae of the southwestern corn borer, *Diatraea grandiosella*. J Comp Physiol B 156, 783 (1986).
23. Gavilanes JG, Lizarbe MA, Municio AM, Oñaderra M: Fluorescence studies on the lipoprotein complex of the fatty acid synthetase from the insect *Ceratitis capitata*. Biochem 20, 5689 (1981).