Fenitrothion-Induced Structural and Functional Perturbations in the Yolk Lipoproteins of the Shrimp *Macrobrachium borellii*

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ABSTRACT: Two lipovitellin (LV) forms containing the same apoproteins but differing in their lipid composition were isolated from *Macrobrachium borelii* eggs at early (LVe) and late (LVl) embryogenic stages and characterized. These two forms of LV, as well as liposomes prepared with lipids extracted from them, were used as simpler models to study the effect of the pesticide fenitrothion (FS) on their structures and functions. Rotational diffusion and fluorescence lifetime of two fluorescent probes [1,6-diphenyl-1,3,5-hexatriene (DPH) and 3-(*p*-(6-phenyl)-1,3,5-hexatrienal)phenylpropionic acid (DPH-PA)] were used to obtain information on structural changes induced by FS in the inner and outer regions of the LV, respectively. Comparison of the rotational behavior of these probes in native LV and liposomes (LP) from extracted LV lipids suggests that apoprotein–lipid interactions result in an ordered neutral lipid core. FS increased the lipid phase polarity of both LV and LP forms. The rotation of these probes in LP was not affected, suggesting a dependence of FS action on lipid–protein interactions. DPH-PA steady-state anisotropy showed that, unlike the LVe form, the LVl form was sensitive to extremely low FS concentrations. The ability of both LV to transfer palmitic acid to albumin was increased, but in a dissimilar manner, by the presence of FS. Such differences in the sensitivity of the LV at different steps of embryogenesis to FS influence the toxic action of this insecticide.

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Pesticides frequently have toxic effects on nontarget organisms. In this regard, some lipophilic insecticides tend to accumulate in cellular membranes, modifying their physicochemical properties and physiological functions (1–3). Small amounts of organophosphate insecticides alter several properties of artificial phospholipid bilayers and natural vertebrate or invertebrate membranes, such as permeability, lipid order, and dynamics (4–12). We have demonstrated that the organophosphate insecticide fenitrothion (FS; *O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate) also incorporates into several circulating invertebrate lipoproteins, altering their lipid dynamics, the penetration of water into the lipid phase, and their ability to exchange FA. Differential alterations in these physical properties were found in shrimp lipoproteins with different lipid and apoprotein compositions (13). These changes were also found in spider lipoproteins, e.g., a different basal lipid order as well as lipid/apoprotein ratio (14). Use of lipoprotein systems with fewer variables should be suitable for carrying out studies of liposomes (LP) or lipoproteins that exhibit either a different lipid or apoprotein composition.

Crustacean lipovitellins (LV), the main energy and carbon source in the vitellus, are necessary for embryogenesis. They are likely to be exposed to insecticides, either by direct water–egg contact or when transported to the ovary by a plasma vitellogenin (15). The shrimp *Macrobrachium borellii* contains only one LV, which is consumed during embryo development (16). In the present work two forms of this LV, one obtained from eggs at early embryonic stages (LVe) and the other at late stages (LVl), were characterized with respect to their lipid and protein compositions. These two forms of LV, as well as LP prepared with lipids extracted from them, were used as simpler models to study the effect of the pesticide FS on their structures and functions.

The structural organization of LV as related to the presence of FS was studied by measuring the lipid-phase dynamics and polarity. The rotational behavior and fluorescent lifetime of two lipid-soluble fluorescent probes, 1,6-diphenyl-1,3,5-hexatriene (DPH) and its propionic acid derivative 3-(*p*-(6-phenyl)-1,3,5-hexatrienyl)phenylpropionic acid (DPH-PA), were determined. Although these probes contain the same fluorescent moiety, they are expected to locate in different regions of the lipoprotein lipid phase. The neutral DPH senses the deep hydrophobic region of lipid bilayers and the neutral lipid core in spherical lipoproteins (17), whereas the amphipathic DPH-PA aligns its carboxylate group with the phospholipid polar head and thus senses the surface lipid monolayer of spherical lipoproteins or the most external regions of lipid bilayers. The same measurements were made in LP prepared with extracted LV lipids to elucidate the role of LV lipids and apolipoproteins.

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Abbreviations: CHO, cholesterol; △, polarized phase shift; DPH, 1,6-diphenyl-3,5-hexatriene; DPH-PA, 3-(*p*-(6-phenyl)-1,3,5-hexatrienyl)phenylpropionic acid; FS, fenitrothion (*O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate); LP, liposome; LPe, liposome at early embryogenic stage; LPl, liposome at late embryogenic stage; LV, lipovitellin; LVe, lipovitellin at early embryogenic stage; LVl, lipovitellin at late embryogenic stage; r_s, steady-state anisotropy; r_∞, limiting anisotropy; SM, sphingomyelin; τ, fluorescence lifetime; τ_m, modulation lifetime; τ_p, phase-shift measured lifetime; τ_r, rotational correlation time.

The importance of FS–lipoprotein interactions can be considered in two ways: (i) Lipoproteins may carry FS to target organs (13), and (ii) FS may alter the transfer of other lipids by the lipoprotein. The latter mechanism was explored in this work by studying the effect of FS on the palmitic acid transfer capacity of LV by using albumin as the acceptor.

EXPERIMENTAL PROCEDURES

Samples. Wild animals were captured in a water course close to Rio de la Plata, Argentina, immediately before the experiment. Eggs from females (150 eggs each) at early (<26 d) and late (>40 d) embryonic development stages were collected. The different stages were identified according to previous reports dealing with the whole embryogenesis (18). Eggs were homogenized in 3 mL of phosphate buffer, 50 mM, pH 7.4, and sequentially ultracentrifuged at 10,000 × *g* for 20 min and then at 100,000 × *g* for 60 min. LV were isolated from the final supernatant (cytosolic fraction).

LV isolation. Yolk lipoproteins were isolated by density gradient ultracentrifugation. Cytosolic fraction (1 mL) was overlayered on 3 mL NaBr solution (density 1.26 g/mL) containing 0.01% sodium azide and centrifuged at 178,000 × *g* for 24 h at 10°C in a Beckman L8 70 M centrifuge, using a SW 60 Ti rotor. Saline solution of the same density as that of samples was centrifuged in parallel to determine relative densities and to check the appropriate gradient formation. Density was determined in a Bausch & Lomb refractometer. The total volume of the tubes was fractionated from top to bottom into 0.2-mL aliquots, and the protein content of each fraction was monitored spectrophotometrically at 280 nm. The zone in the gradient containing LV (aliquots 9–11) was separated as a whole fraction. Thus, LVe and LVl were obtained from eggs at the early and late embryogenesis stages, respectively.

Lipid and protein analysis. Lipids were extracted following the method of Folch *et al.* (19). Lipid classes were quantified by TLC coupled with an FID in an Iatroscan apparatus Model TH-10, after separation on Chromarods SIII (Iatron, Tokyo, Japan), using a triple development solvent system as described previously (20). MAG was used as internal standard. FA were analyzed by GLC under the conditions described previously (21).

Total protein concentration in each fraction isolated from the density gradient was measured colorimetrically by the method of Lowry *et al.* (22). LVe and LVl apoproteins were analyzed by native and dissociating electrophoresis. Analyses in nondissociating conditions were performed by using a 4–23% PAGE. Protein subunits were analyzed by SDS-PAGE using a gradient of 4–23% acrylamide (23). The gels were stained with Coomassie Brilliant Blue R-250 (Sigma Chemical Co., St. Louis, MO). M.W. were calculated as previously described (24).

LP preparation. Total lipids of LVe and LVl were extracted and used for LP preparation (LPe and LPl, respectively). Extracts containing 1 mg lipids were evaporated to dryness, hydrated with 3 mL of 50-mM potassium phosphate buffer, pH 7.4, vortexed, and then sonicated using a tip sonicator for 5 min. After centrifugation for 2 h at 80,000 × *g*, lipid analysis of the supernatant confirmed that all lipids originally present in the sample had been dispersed and incorporated into LP.

Sample labeling with fluorescent probes. For labeling, 3 mL of 50-mM potassium phosphate buffer, pH 7.4, with LV (0.1 mg/mL in protein) or LP (0.09 mg/mL in lipid) were mixed with a few microliters of concentrated DMSO solutions of DPH or DPH-PA to reach a final concentration of 2 μM. Blanks without the fluorescent probes and with the same volume of DMSO were used to correct the measurements for nonspecific fluorescence and light scattering. Samples were gently swirled at 20°C for at least 2 h in darkness to allow a complete equilibration of the probes with the LV or LP. FS from a concentrated ethanolic solution was added to samples prior to equilibration at concentrations of 1, 10, or 20 ppm.

Fluorescence measurement. All fluorescence measurements were carried out in an SLM 4800 C phase-modulation spectrofluorometer (SLM Instruments Inc., Urbana, IL). Steady-state anisotropy (r_s) was measured within a temperature range of 10–30°C for LV, and 10–45°C for LP. Fluorescence lifetime (τ) and differential polarized phase shift (△) were measured only at 10 and 30°C.

Measurements of r_s, τ, and △ were made as described (12) by using an excitation wavelength of 361 nm and a cutoff filter (Schott KV 389) to isolate the emitted light. For τ measurements, exciting light was amplitude-modulated at 18 and 30 MHz by a Debye–Sears modulator and vertically polarized by a Glan–Thompson polarizer. Emitted light passed through the filter and then through a Glan–Thompson polarizer oriented 55° to the vertical to eliminate effects of Brownian motion (25). Phase shift and demodulation of emitted light relative to a reference of known τ were determined and used to compute phase-shift measured lifetime (τ_p) and modulation lifetime (τ_m), respectively (25). 1,4-Bis(5-phenyloxazol-2-yl) benzene in ethanol, which has a τ of 1.35 ns (26,27), was used as reference. △ was determined according to Lakowicz (28,29) by excitation with light modulated at 18 and 30 MHz and vertically polarized, and the phase difference between the parallel and perpendicular components of the emitted light was measured.

The obtained values for r_s, τ, and △ were used to calculate the limiting anisotropy (r_∞) and the rotation rate as previously described (26,27) in accordance with the theory developed by Weber (30). Calculation of τ_r and r_∞ from measurements at discrete frequencies requires homogeneity in the rotamer fluorescence lifetime (25,26).

Release of FA from LV. Proteins from LVe and LVl (30 mg) were incubated with 3 μCi [1-^{14}C]palmitic acid (57.0 mCi/mmol, 99% radiochemically pure; NEN, Boston, MA) as the ammonium salt for 30 min and dialyzed for 24 h using 0.1 M Tris-HCl, pH 8.0, to remove the nonbound FA. The labeled LVe and LVl were exposed to 0, 1, and 20 ppm of FS for 1 h. Samples were then incubated with 20 mg/mL of albumin as FA acceptor for 30 min. After incubation, samples were analyzed under native conditions by preparative gel filtration fast-flow protein LC on a Superdex 200 HR 10/30 column (Amersham-Pharmacia, Uppsala, Sweden) using 0.1 M Tris-HCl, pH 8.0,

at a flow rate of 0.4 mL/min. Protein was detected spectrophotometrically at 280 nm. The column was calibrated with thyroglobulin, ferritin, BSA, and ribonuclease A (Amersham-Pharmacia) as M.W. protein markers. LV and albumin fractions were separately collected on the basis of their retention times and relative mass ratios. The radioactivity of palmitic acid bound to each protein peak was quantified by liquid scintillation counting. The amount of protein in each peak was colorimetrically quantified using the method of Lowry *et al.* (22).

RESULTS

Isolation and characterization of LV. LV were isolated from the cytosol of eggs at different embryonic development stages by density gradient ultracentrifugation. Measurements of absorbance at 280 nm performed on each fraction from the gradients showed similar protein profiles for both LV forms (Fig. 1). Fractions containing the maxima, corresponding to LVe and LVl, respectively, were characterized separately. Hydrated density, and lipid and FA compositions of both forms of LV are shown in Table 1. PC was the most abundant lipid in LVe, followed by other phospholipids and TAG. A marked decrease in PC, a minor decrease in TAG, and a concomitant increase in FFA, cholesterol (CHO), and sphingomyelin (SM) contents were observed throughout development. Also, palmitic acid (16:0) declined as the percentage of 20-carbon PUFA (20:4 and 20:5) increased. Lipid/protein ratios in LVe and LVl were found to be similar and consistent with their densities.

Figure 2 shows the results obtained from the electrophoretic analysis of LVe and LVl performed under native (A) and dissociating (B) conditions. Electrophoretic mobility of the proteins revealed, under native conditions, a predominant band of 440 kDa in both LV (Fig. 2A). In dissociating conditions, two bands of 94 and 112 kDa were observed (Fig. 2B).

Effect of FS on the rotational behavior of DPH and DPH-PA in LV and LP built up with LV lipids. Steady-state anisotropy of DPH (Figs. 3 and 5) and DPH-PA (Figs. 4 and 6) were measured over temperature ranges of 10–30°C in LV (Figs. 3 and 4) and 10–45°C in LP (Figs. 5 and 6) in the absence or the presence of 1, 10, and 20 ppm of FS. Although r_s is often used as a mobility parameter, it is highly dependent on τ. Then measurements of τ are essential to interpret r_s variations correctly and to determine the environment polarity. Time-resolved or phase-modulation-resolved anisotropy as limiting anisotropy (r_∞) and rotational correlation time (τ_r) were used for a thorough interpretation of the rotational motion of a fluorescent probe (DPH or DPH-PA) and the environment properties. r_∞ is inversely related to the extent of wobbling motion of the probe

TABLE 1
Lipid Composition[a] of Lipovitellins Isolated from Vitellus of Egg Yolk of *Macrobrachium borellii*

	Fraction	
Lipid classes	LVe	LVl
TAG (%)	20.5 ± 1.8	16.7 ± 1.5
FFA (%)	5.9 ± 0.6	10.4 ± 0.7
Cholesterol (%)	7.9 ± 0.5	13.7 ± 1.0
PE (%)	15.8 ± 1.2	19.5 ± 3.6
PC (%)	41.9 ± 3.6	24.9 ± 4.4
Sphingomyelin (%)	7.6 ± 0.5	14.1 ± 1.5
Major FA (%)		
14:0	3.7	2.4
16:0	22.3	17.6
16:1n-7	14.0	10.9
18:0	9.0	8.9
18:1n-9	20.1	20.5
18:1n-7	10.6	11.9
18:2n-6	7.0	7.7
20:4n-6	4.1	7.0
20:5n-3	7.8	11.9
Total lipids (mg/150 eggs)	14.1 ± 4.3	3.5 ± 2.6
Total proteins (mg/150 eggs)	47.2 ± 8.0	14.1 ± 7.0
Lipid/protein ratio	0.29	0.25
Hydrated density (g/mL)	1.18–1.19	1.18–1.19

[a]Data are expressed as weight percentage of lipids as determined by TLC-FID. Values represent the mean ± SD of three analyses. LVe, lipovitellin at an early embryogenic stage; LVl, lipovitellin at a late embryogenic stage.

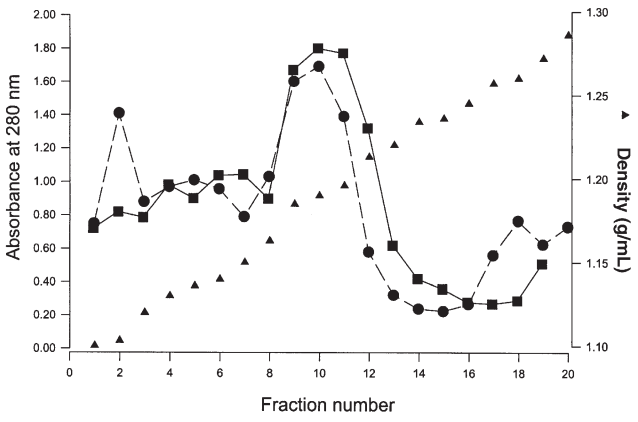

FIG. 1. Total protein (absorbance at 280 nm) and density distribution (▲) in egg cytosol fractions after gradient ultracentrifugation. Samples of egg cytosol were obtained at early (LVe) (●) and late (LVl) (■) stages of embryonic development. LVe, lipovitellin at an early embryogenic stage; LVl, lipovitellin at a late embryonic stages.

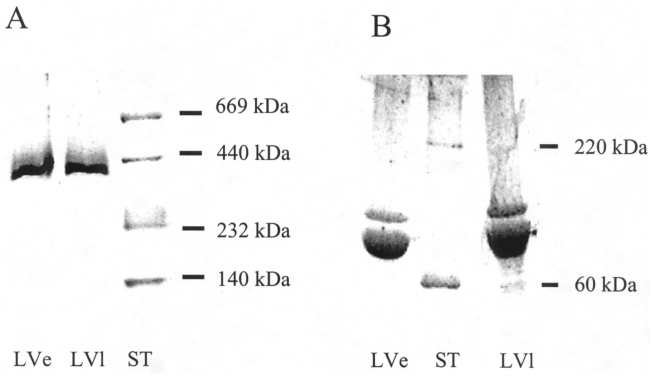

FIG. 2. Native (A) and dissociating (B) gel electrophoresis of LVe and LVl. Both gels were done using polyacrylamide gradients of 4–23% wt/vol. Proteins were revealed by Coomassie Blue staining. ST, standard; for other abbreviations see Figure 1.

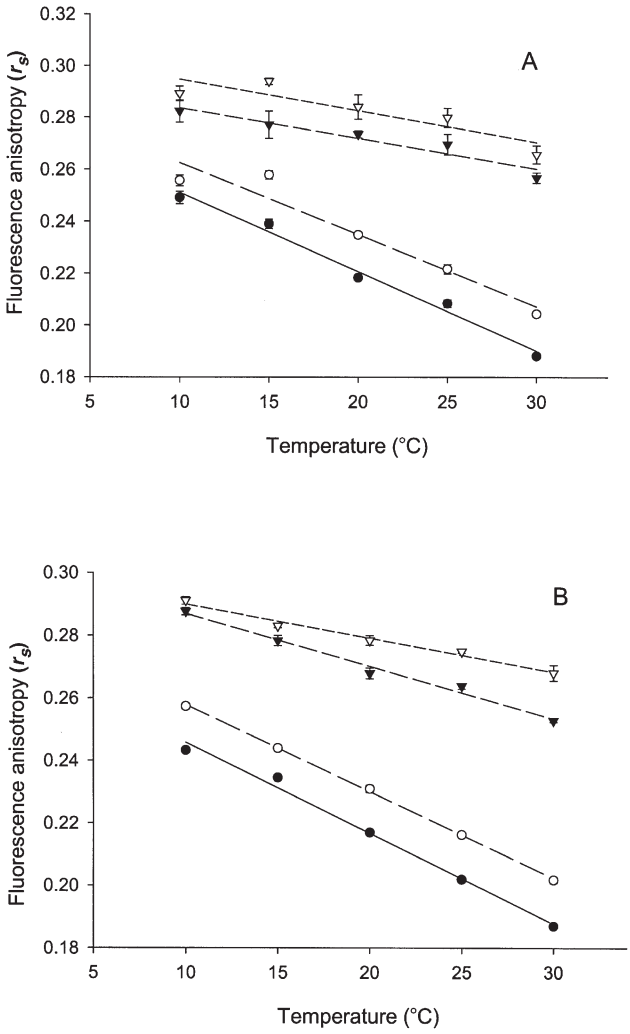

FIG. 3. Effect of fenitrothion (FS) on the 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence anisotropy (r_s) in LVe (A) and LVl (B). r_s is plotted vs. temperature in the absence (●) or in the presence of 1 (○), 10 (▼), and 20 ppm (▽) of FS. Values represent the average of five different determinations ± SD. For other abbreviations see Figure 1.

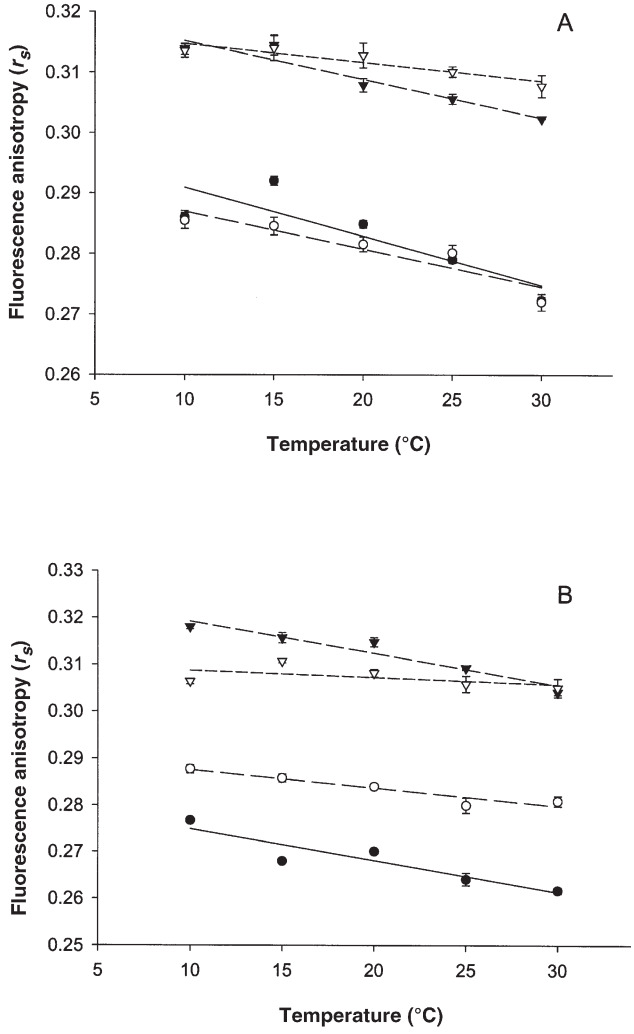

FIG. 4. Effect of FS on the 3-(*p*-(6-phenyl)-1,3,5-hexatrienyl)phenylpropionic acid (DPH-PA) r_s in LVe (A) and LVl (B). (r_s) is plotted vs. temperature in the absence (●) or in the presence of 1 (○), 10 (▼), and 20 ppm (▽) of FS. Values represent the average of five different determinations ± SD. For other abbreviations see Figures 1 and 3.

and indicates the ordering of the environment; τ_r is the inverse of the rotational rate and provides data on the environmental viscosity. For both probes, but especially for DPH, τ_m were somewhat higher than phase lifetimes, τ_P, indicating some heterogeneity in the fluorophore population. However, the fact that the values obtained for τ_r and r_∞ were relatively independent of the frequency indicated that they were essentially correct average values of the rotamer populations.

(*i) Influence of apolipoproteins and different lipid composition on LVe and LVl.* All samples showed a linear decrease of r_s with temperature, but DPH r_s showed a greater temperature dependence than did DPH-PA r_s (Figs. 3–6). Comparison of LV with their corresponding LP indicated that the presence of apolipoproteins increased the r_s in both probes, this effect being more conspicuous for DPH r_s. This particular effect was also observed on τ values, since apolipoproteins increased the lifetimes of DPH without altering DPH-PA lifetimes (Fig. 7). r_∞ was increased by apolipoproteins in the environment of DPH-PA (up to 2.5-fold higher at 30°C), and to an even greater extent in the environment of DPH (up to 12-fold higher in LV than in LP) (Table 2). Moreover, the time τ_r of DPH was increased by the presence of apolipoproteins, but DPH-PA τ_r was not affected (Table 2). Altogether, these data indicate a particular effect of apolipoproteins on the neutral lipid core, decreasing the DPH environment polarity as well as hindering and slowing the DPH rotational motion. In the absence of apolipoproteins, DPH rotation became nearly isotropic (with a very low r_∞) at 30°C.

Similar DPH r_s values were observed when comparing both LV, whereas DPH-PA r_s was higher in LVe than in LVl (Fig. 4). A similar difference was observed when comparing LPe with LPl (Fig. 6); a somewhat higher DPH-PA r_∞ was observed in

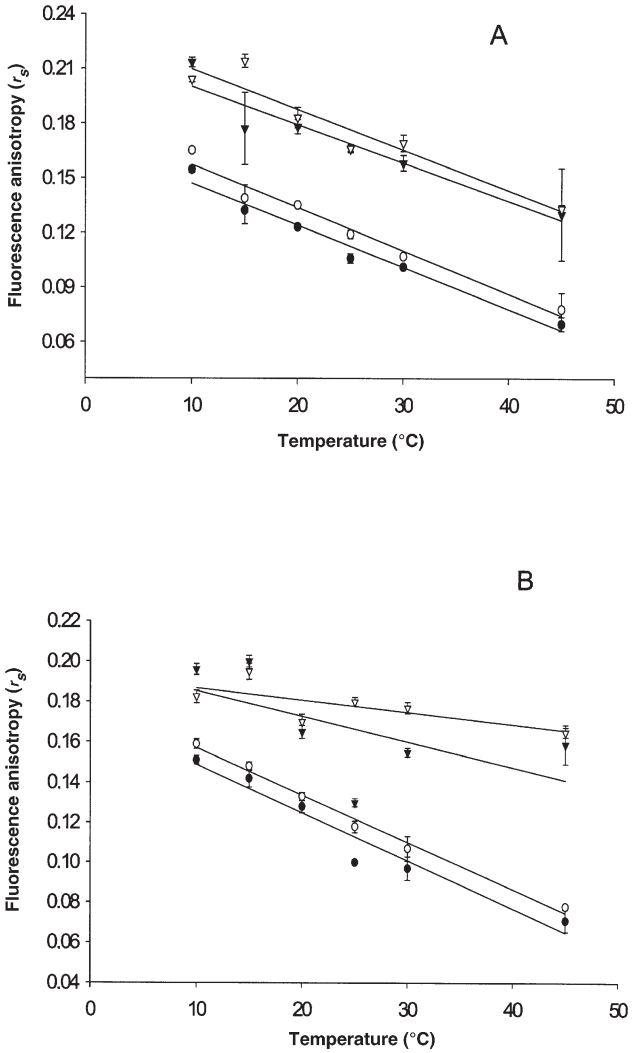

FIG. 5. Effect of FS on the DPH r_s in LPe (A) and LPl (B). r_s is plotted vs. temperature in the absence (●) or in the presence of 1 (○), 10 (▼), and 20 ppm (▽) of FS. Values represent the average of five different determinations ± SD. For abbreviations see Figures 1 and 3.

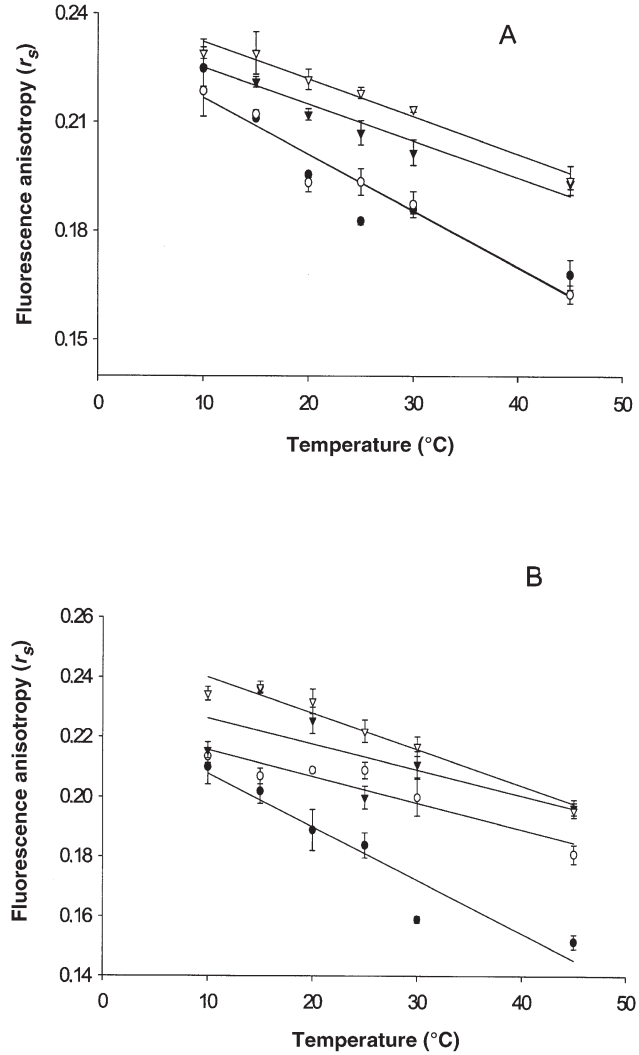

FIG. 6. Effect of FS on the DPH-PA r_s in LPe (A) and LPl (B). r_s is plotted vs. temperature in the absence (●), or in the presence of 1 (○), 10 (▼), and 20 ppm (▽) of FS. Values represent the average of five different determinations ± SD. For abbreviations see Figures 1, 3, and 4.

LVe with respect to LVl (see Table 2), and the same trend was observed when comparing LPe with LPl. However, no differences could be found in r_∞ of DPH nor in the τ_r of DPH and DPH-PA in both LV forms. These facts suggest that the different lipid compositions of the LV affected the lipid ordering in the surface monolayer but not in the neutral lipid core. A slightly increased τ for both probes also was observed in LVe with respect to LVl (Fig. 7). However, differences in τ values were not noticeable when comparing LPe with LPl in the absence of apolipoproteins (Fig. 7); they could be attributed to a different interaction of apolipoproteins with the lipids in each LV.

(ii) Influence of FS. The presence of high concentrations of FS resulted in a strong increase of r_s for both probes in both types of LV and LP. The major effect of the insecticide was observed at higher temperatures, resulting in a decreased temperature dependence of r_s in FS-containing samples. FS also produced a large decrease in τ of both probes, an effect that was greater with DPH (Fig. 7). No detectable effect of FS on the rotational behavior of DPH and DPH-PA in the LP samples could be observed. Thus, the increase in DPH and DPH-PA r_s that was noted in samples containing FS was exclusively due to the reduction of τ. The same effect was observed in LVe for DPH-PA, whose rotational behavior was not affected by FS (Table 2). But an increase in lipid ordering, as reflected by measurements of r_∞ values, was detected for both LV with the core-sensing probe DPH, and only for LVl with the surface probe DPH-PA (see Table 2). A decrease in τ_r of DPH and DPH-PA was also observed for LVl, but not for LVe. Thus, these data indicated that FS was incorporated into all these systems, decreasing the polarity of the regions sensed by DPH and DPH-PA. However, FS altered the lipid dynamics only in the neutral lipid core sensed by DPH in LVe, whereas both

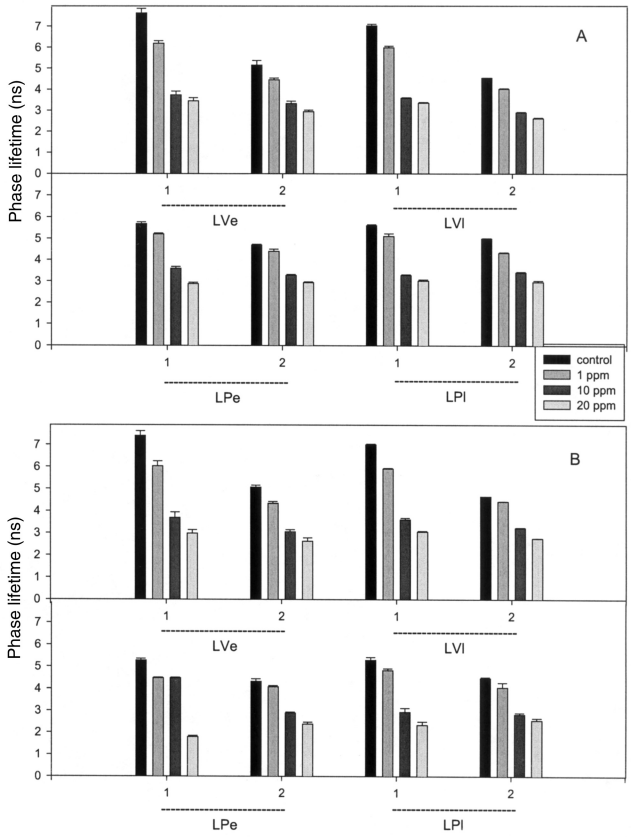

FIG. 7. Phase lifetime (τ_P) of DPH (1) and DPH-PA (2) in LVe, LVl, LPe, and LPl of *Macrobrachium borellii*, measured at 18 MHz in the absence or presence of 1, 10, and 20 ppm FS at 10 (A) and 30°C (B). Student's *t*-test was used to compare the significance of the differences with respect to the sample without FS: $P < 0.0001$. For abbreviations see Figures 1, 3, and 4.

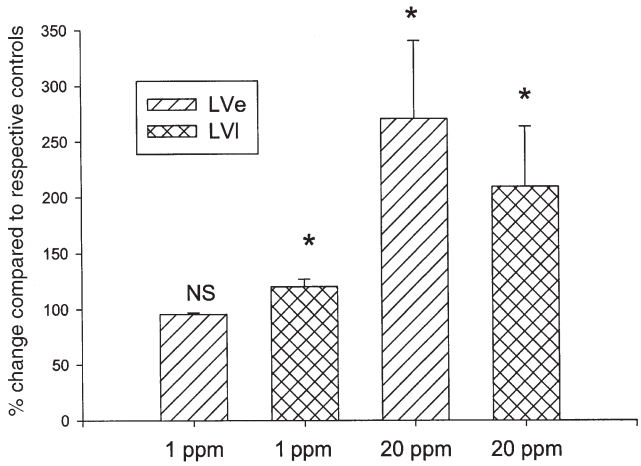

FIG. 8. Release of palmitic acid from LVe and LVl. Releasing capacity of palmitic acid from LV of *M. borellii* was checked in the presence of 1 and 20 ppm of FS. Purification of LV was done by FPLC, and radioactivity was quantitated by liquid scintillation. *P* was calculated by Student's *t*-test by comparison of treated and untreated samples: $P < 0.05$(*); NS, not significant.

regions (neutral core and surface monolayer) were affected in LVl. These effects of FS on the lipid dynamics were dependent on the presence of apolipoproteins, since they were not evident in the protein-free LP systems.

At low FS concentrations, r_s measurements for LV and LP showed different degrees of sensitivity to the insecticide. On one hand, the lipid region sensed by DPH was more sensitive to 1 ppm FS in whole LV (Fig. 3) than in LP (Fig. 5), also pointing out the importance of apolipoproteins on the FS action in the neutral lipid core. On the other hand, DPH-PA r_s was sensitive to 1 ppm FS in LVl but not in LVe (Fig. 4), and the same was observed for the corresponding LP (Fig. 6), suggesting that the different lipid compositions of these lipoproteins can modulate the effect of FS on the surface lipid monolayer properties.

Effect of FS on the transfer of [^{14}C]palmitic acid from LV to albumin. Figure 8 shows the percentage of [^{14}C]palmitic acid transferred from LV to albumin either in the absence or in the presence of 1 and 20 ppm of FS. The addition of 20 ppm FS produced an increment of around 170 and 109% in palmitic acid transfer from LVe and LVl, respectively. However, at low FS concentrations, LVl was more sensitive than LVe, since 1 ppm FS did not alter palmitate transfer from LVe but increased it around 20% from LVl.

TABLE 2
Rotation Correlation Time (τ_r) and Limiting Anisotropy (r_∞) of DPH and DPH-PA at 30°C in Liposomes (LP) and LV[a]

		τ_r	r_∞			τ_r	r_∞
DPH							
LVe	Control	2.40 ± 0.6	0.12 ± 0.02	LVl	Control	2.20 ± 0.26	0.12 ± 0.001
	+20 ppm	1.60 ± 1.0	0.21 ± 0.05*		+20 ppm	1.30 ± 0.70 *	0.22 ± 0.02*
LPe	Control	1.38 ± 0.4	0.02 ± 0.03	LPl	Control	1.50 ± 0.60	0.01 ± 0.04
	+20 ppm	2.24 ± 2.0	0.01 ± 0.05		+20 ppm	1.40 ± 0.69	0.08 ± 0.05
DPH-PA							
LVe	Control	1.80 ± 1.0	0.23 ± 0.02	LVl	Control	1.87 ± 0.24	0.2 ± 0.008
	+20 ppm	1.88 ± 1.0	0.25 ± 0.06		+20 ppm	0.94 ± 0.50*	0.28 ± 0.01*
LPe	Control	1.63 ± 0.8	0.10 ± 0.04	LPl	Control	1.60 ± 0.57	0.08 ± 0.04
	+20 ppm	0.90 ± 0.4	0.16 ± 0.02		+20 ppm	1.20 ± 1.0	0.14 ± 0.07

[a]Values represent the average of five determinations ± SD. *P* was calculated by two-way Student's *t*-test, comparing samples with their respective controls. Asterisk (*), $P < 0.001$. DPH, 1,6-diphenyl-3,5-hexatriene; DPH-PA, 3-(*p*-(6-phenyl)-1,3,5-hexatrienyl)-phenylpropionic acid; for other abbreviations see Table 1.

DISCUSSION

Structural organization of lipids in LV. Neutral and amphipathic probes like DPH and DPH-PA have been used to obtain information on the structural organization of natural (17) or reconstituted (31) lipoproteins. In lipoproteins with a high content of neutral lipids such as TAG, these lipids build up a central core surrounded by a superficial monolayer of amphipathic lipids. Certain lipids such as DAG (17) and CHO (32) can be distributed between the core and surface. On the other hand, lipoproteins lacking neutral lipids have a discoidal morphology with a bilayer of amphipathic lipids (33). LV lack cholesteryl esters but have about 20% of TAG that are, in principle, a somewhat high amount to be easily accommodated within a phospholipid bilayer. It is expected that a neutral molecule such as DPH will display less hindered rotation in a disordered neutral lipid core than in a lipid bilayer. Although r_∞ of DPH in LV is lower than that of DPH-PA, it is relatively high for a disordered lipid core and compatible with values obtained in lipid bilayers. However, it should be taken into account that DPH should partition between the core and superficial monolayer (17), and a relatively small core has to be expected with only 20% of neutral lipids. Another fact to be taken into account is the influence of apolipoproteins. In this respect, the results obtained here with LP of LV lipids are enlightening. DPH r_∞ is extremely low in these protein-free systems, indicating nearly isotropic rotation, which is incompatible with a bilayer organization. This fact suggests that, in the absence of apolipoproteins, LV lipids are organized as small emulsions with a highly disordered core and a surface monolayer of amphipathic lipids. Comparison of the rotational behavior of DPH and DPH-PA in LV and LP systems indicates that apoproteins preferentially hinder DPH rotation. It is possible that apolipoproteins force the incorporation of neutral lipids of LV into the bilayer of a discoidal lipoprotein. However, recent evidence obtained from negative staining electron microscopy (Garcia, C.F., unpublished results) indicates that LVe does not form the "rolls" of stacked structures characteristic of discoidal lipoproteins. Thus, TAG in LV likely form a core with relatively high ordering owing to interaction with one or both apolipoproteins. This interaction of apolipoproteins with the neutral lipid core of LV is also suggested by the selective increase in DPH τ, when comparing LV with LP systems.

LV modification during embryogenesis. During embryogenesis in *M. borellii*, the lipid composition of LV was modified without changes in apolipoprotein composition and lipid/protein ratio. The major changes were an increase in SM, CHO, and FFA with a corresponding decrease in PC contents. A replacement of saturated FA by PUFA was also observed. One of the aims of this work was to study how these changes in lipid composition affect the lipid dynamics and apolipoprotein–lipid interaction in LV. In spite of the large changes in lipid composition in LVe and LVl, no difference in the rotational mobility of DPH was observed, and only a small decrease in lipid ordering was sensed by DPH-PA in LVl compared with LVe. The environmental polarity of both probes was somewhat higher in LVl than in LVe. Moreover, these differences between LVe and LVl were not evident when the apolipoprotein-free systems LPe and LPl were compared, indicating that they depended on apolipoprotein–lipid interactions or some other structural characteristic that was disrupted in the procedure of lipid extraction and LP preparation. These results indicated that the condensing effect expected as a result of the increase in CHO and SM content in LVl was counterbalanced by other changes such as replacement of saturated FA by PUFA or the increase in FFA content.

Effect of FS on LV lipid phase properties. FS at high concentrations decreased the τ of DPH by more than 50% and of DPH-PA by about 30% in both LV and LP. This effect was observed in other lipoprotein and lipid systems (13,14) and was attributed to an increased amount of water penetration in the lipid phase owing to packing defects produced by the insecticide. FS also produced an increase in r_∞ of DPH in LVe and LVl, indicating that this insecticide increased the ordering of the neutral lipid core in both LV. The rotational behavior of DPH-PA, however, was affected by FS in LVl but not in LVe, a difference that should be attributed to the different compositions in amphipathic lipids evoked by these forms of LV. FS affected the properties of the superficial monolayer of LVl in such a way that DPH-PA seemed to wobble in a more restricted angle but at a higher rate, as indicated by the increase in r_∞ and the decrease in τ_r. No appreciable influence of FS on the rotational behavior of DPH or DPH-PA was observed in LP of the extracted LV lipids, a fact that indicates that apolipoprotein–lipid or apolipoprotein–FS interactions play an important role in the effect of this insecticide. A higher sensitivity of LVl to FS in comparison with that of LVe is evidenced by measurements of DPH-PA r_s at low FS concentrations (1 ppm).

Effect of FS on the transfer of palmitic acid to albumin. The ability of LV to transfer palmitic acid to albumin is increased by FS in a concentration-dependent way. It was shown that FS decreased FA uptake by other lipoproteins (13). These facts suggest that FS decreases the capacity and/or affinity of lipoprotein to bind FA, which in turn might be due to a competition between FS and FA for the same binding site or to the alteration of the lipid phase properties produced by FS. Further experimentation will be necessary to distinguish among these possibilities and to identify the molecular basis of FS action on the ability of LV to transfer FA. At a low FS concentration (1 ppm), palmitic acid transfer to albumin was not affected in LVe, but it was increased about 20% in LVl; this fact correlates with a higher sensitivity of LVl to the changes produced by FS in the lipid phase properties.

In short, these results indicate that the modification in lipid composition during embryogenesis in *M. borellii* LV plays an important role in the structural changes and alterations in FA transfer produced by FS. Such differences in sensitivity to FS of the LV at different steps of embryogenesis affect the toxic action of this insecticide. Additional research will be required to learn whether FS can alter the transference of FA or other lipids to physiological acceptors.

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