

LOCUST ADIPOKINETIC HORMONE STIMULATES LIPID
MOBILIZATION IN MANDUCA SEXTA

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Adipokinetic hormone, a decapeptide isolated from the locust, stimulates mobilization of diacylglycerols from the locust fat body and loading of the lipid transport protein, lipophorin. Injection of the synthetic locust adipokinetic hormone into a sphinx moth, Manduca sexta, causes lipid loading of lipophorin. The lipophorin decreases in density from 1.11 to 1.06 g/ml, and a soluble protein from the hemolymph (apolipophorin III) associates with the lipophorin particle. Administration of intermediate doses of hormone indicates that lipophorin is converted directly to the low density form; no appreciable amounts of intermediate density particles are formed.

Most insects have primarily a single type of lipoprotein (lipophorin) in the blood (hemolymph) which seems to serve the multiple functions of the family of mammalian lipoproteins (1). Most lipophorins so far examined have a predominance of phospholipids and diacylglycerols (2). There are generally two apoproteins, one of high and the other of moderate molecular weight (~250,000 daltons and ~80,000 daltons (3)). In adult locusts, an additional small apoprotein appears to associate reversibly with lipophorin (4-7).

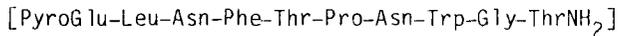
The larva of Manduca sexta has a homogeneous lipophorin of density 1.13 g/ml, with 40 percent lipid and 60 percent protein; the latter consists of two apoproteins, apolipophorin I (apoLp-I; ⁺⁺ 245,000 daltons) and apolipophorin II (apoLp-II; 78,000 daltons) (8,9). In the adult (sphinx moth) stage of M. sexta, we discovered that adult lipophorin can associate with a small soluble protein (~17,000 daltons) in the hemolymph, apolipophorin III (apoLp-III). The adult lipophorin has a lower density (1.11 g/ml) than that of the larva,

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⁺⁺Abbreviations used: ApoLp-I, apolipophorin I; ApoLp-II, apolipophorin II; ApoLp-III, apolipophorin III; AKH, adipokinetic hormone; SDS, sodium dodecyl sulfate.

and in adult males there is an additional form of even lower density (1.06 g/ml) present in small amount.

Adipokinetic hormone (AKH), an N- and C-terminal blocked decapeptide



characterized from the locust, Locusta migratoria, (10) promotes loading of lipophorin in locust hemolymph and association with a small soluble protein (4-7). We have now shown that injection of the locust hormone into adult M. sexta promotes lipid loading and association with apoLp-III.

MATERIALS AND METHODS

Animals. M. sexta eggs were supplied by Dr. J. P. Reinecke and Dr. James Buckner, U. S. Department of Agriculture, Fargo, ND. The animals were reared as previously described (11). Animals 1 to 3 days after adult eclosion were used in experiments.

Injections and Bleeding. Synthetic locust adipokinetic hormone (AKH; Peninsula Laboratories) was dissolved in physiological saline (either phosphate buffered saline (PBS; 0.15 M Na phosphate/0.10 M NaCl/0.05 percent EDTA/pH 7.0) or Cherbas saline (12)), and 50 μ l were injected into a midabdominal intersegmental membrane of unanaesthetized adult males with a 26 gauge needle. After 1 hr, legs, wings, and head were removed and hemolymph bled into 10 μ l of PBS containing 50 mM glutathione and 10 mM diisopropylphosphorofluoridate by centrifuging carcasses head down at 300 rpm.

Ultracentrifugation. Hemolymph from 5 to 7 animals (0.5-1.0 ml) was added to 8.86 g of KBr dissolved in PBS, then diluted with PBS to 20 ml. The 20 ml of solution were overlaid with 0.9 percent NaCl in Beckman Quick-seal tubes and centrifuged in a Beckman L8-70 ultracentrifuge with slow accelerate feature for 4 hr at 10°C. Tubes were fractionated from the top through a Pasteur pipet and interfaced peristaltic pump - fraction collector combination (Pharmacia or Isco). Absorbance of fractions at 280 nm was read in a Perkin-Elmer Lambda 3 spectrophotometer. Densities were calculated from KBr refractivity read on a B&L refractometer.

Electrophoresis. Prior to electrophoresis, samples were dialyzed in a microdialyzer (BRL) against PBS to remove KBr. Sodium dodecyl sulfate (SDS) - polyacrylamide gel electrophoresis (13) employed 4-15 percent gradient gels (18x20x0.15 cm) poured from a gradient maker (BRL). Protein standards were from Bio-Rad.

Protein and Lipid Assays. Fatty acids of samples were measured as copper salts (14), using triolein (Nu Chek Prep) standards, after hydrolysis for 1 hr at 95°C with 1 N KOH in aqueous methanol.

Protein was assayed by the SDS-Folin-phenol method of Peterson (15). Larval lipophorin, showing a specific absorbance of 0.75 $\text{mg}^{-1}\text{ml}^{-1}$ at 280 nm (as estimated by the biuret assay (9)), was used as a standard.

RESULTS AND DISCUSSION

Fig. 1A shows the density profiles of larval and adult male M. sexta proteins. The lipophorin of the adult moth is less dense ($d=1.11$ g/ml) than that

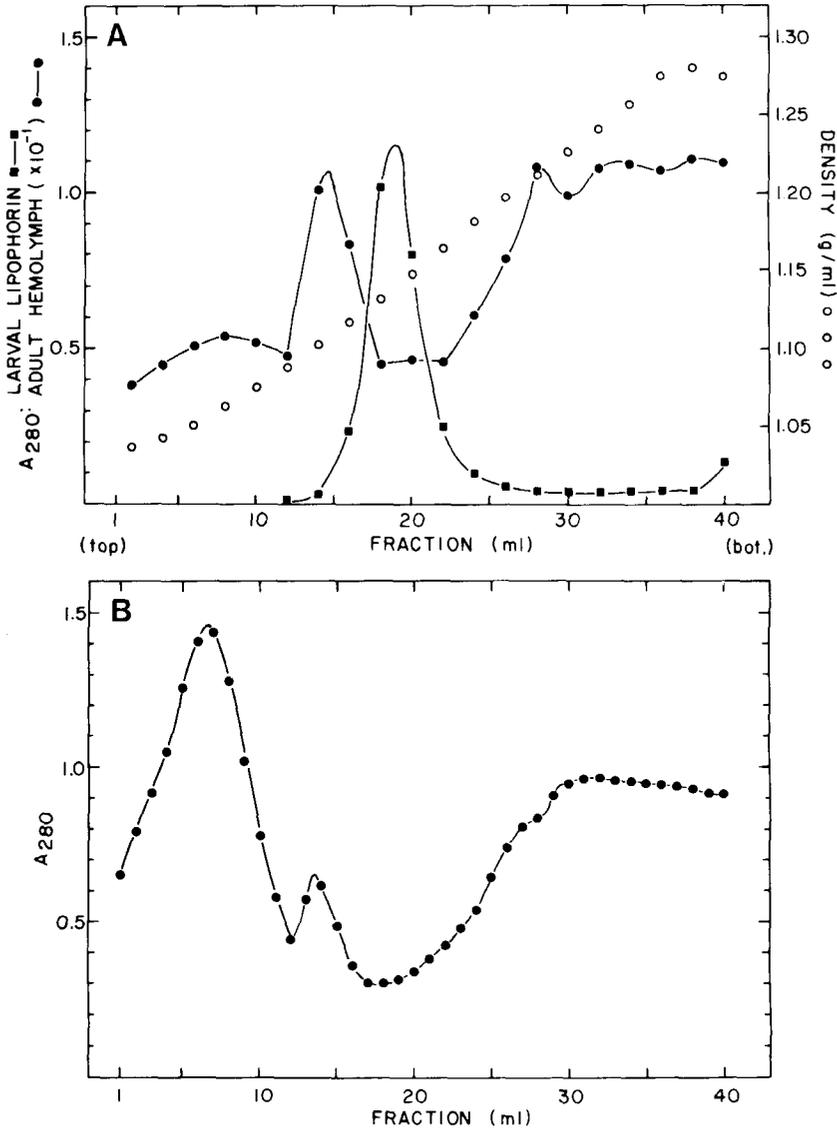


FIGURE 1. Ultracentrifuge density gradient profiles of larval and adult lipophorins. Larval lipophorin (A), hemolymph from adult males injected with saline (A), or hemolymph from adults injected with a saline solution of 200 pmoles AKH (B), was added to KBr solution and overlaid with 0.9 percent NaCl, then centrifuged as described. Tubes were fractionated from the top into 1 or 2 ml fractions and absorbance at 280 nm read. (B) Adults were injected with 200 pmoles of AKH 1 hr before bleeding and centrifugation.

of the larval hornworm ($d=1.13$). One hour following injection of 200 pmoles of synthetic locust adipokinetic hormone into moths, adult lipophorin is dramatically shifted to a lower density (1.06 g/ml; Fig. 1B).

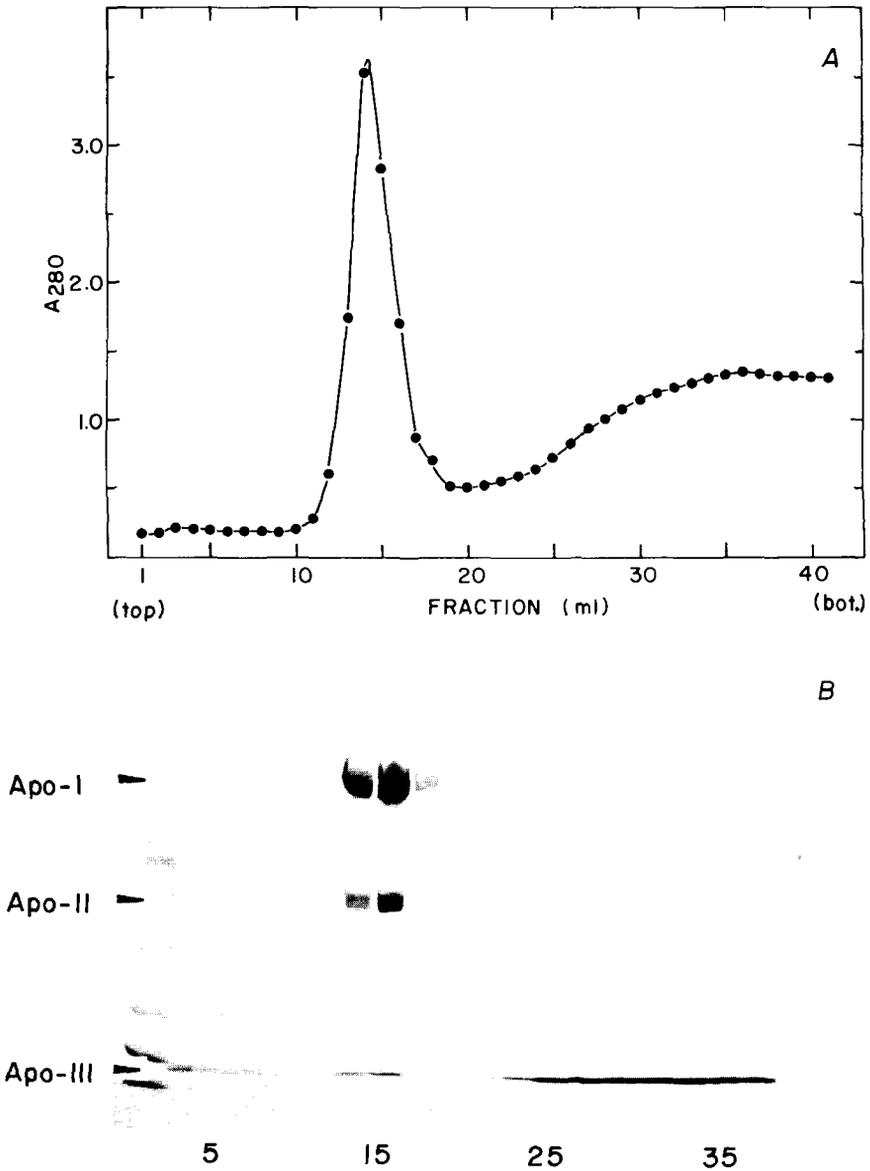


FIGURE 2. Ultracentrifuge density gradient profile of adult hemolymph from 5 males injected with saline (A) and SDS-polyacrylamide gel of density gradient (B). Samples (30 μ l each) were applied in 30 μ l of SDS buffer with β -mercaptoethanol. Left lane, low molecular weight standards (2 μ l): Phosphorylase B (93,000), bovine serum albumin (67,000), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), lysozyme (14,400); Right lane, high molecular weight standards (2 μ l): Myosin (200,000), β -galactosidase (116,000), phosphorylase B, bovine serum albumin, ovalbumin.

Injection of a lower dose of AKH (100 pmoles) results in a partial shift of protein to the lower density peak; no peak of intermediate density appears (compare saline injected controls, Fig. 2, with hormone injected animals, Fig.

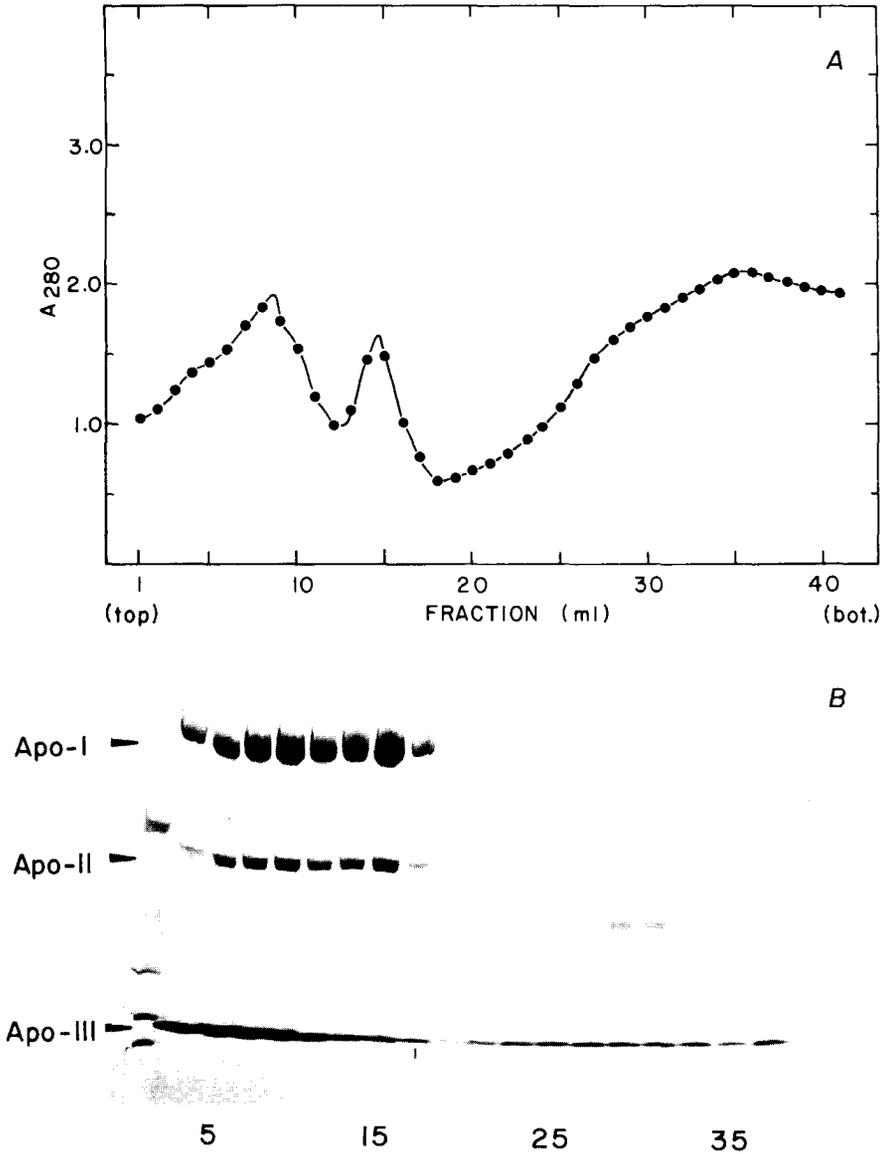


FIGURE 3. Ultracentrifuge density gradient profile of adult hemolymph from 7 males injected with 100 pmoles AKH (A) and SDS-polyacrylamide gel of density gradient (B). Samples and standards (left and right lanes) applied as in figure 2.

3). This suggests that the lipid loading responsible for the density shift is processive, i.e., that a lipophorin particle is fully loaded before it is released from a loading site at the fat body. The processive nature of the loading process may also be a consequence of the lack of stability of particles of intermediate density (16).

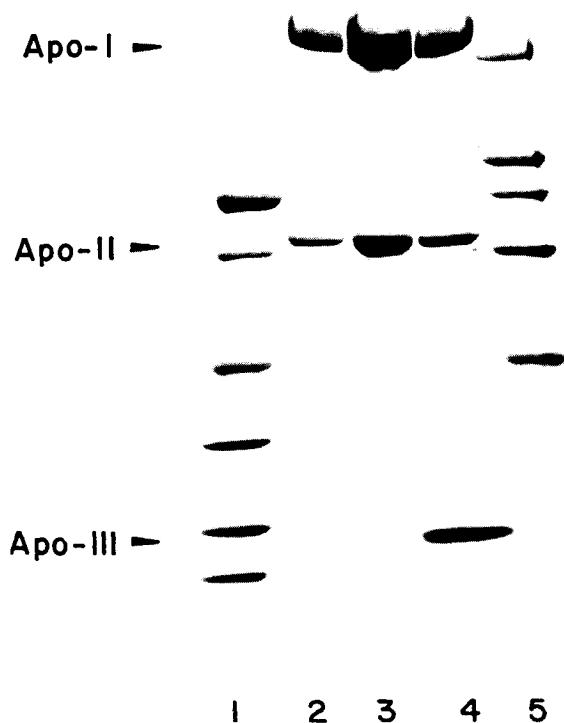


FIGURE 4. SDS-polyacrylamide gel of larval and adult lipophorins. Lane 2, larval lipophorin ($d=1.13$); lane 3, unloaded adult lipophorin ($d=1.11$, from peak fractions 14-15 in figure 3); lane 4, lipid-loaded adult lipophorin ($d=1.06$, from peak fractions 7-9 in figure 3). Lanes 1 and 5, low and high molecular weight standards, respectively, as in figure 2.

SDS gel electrophoresis shows that most of the apoLp-III is free in the subnatant of a density gradient centrifugation of normal adult hemolymph (Fig. 2B). However, following injection of 100 pmoles of AKH, most of the apoLp-III associates with the low density lipophorin near the top of the tube (Fig. 3B). The area of this low density peak (Fig. 3A) is actually underrepresented, since apoLp-III shows a negligible absorbance at 280 nm (17).

SDS-polyacrylamide gel electrophoreses of the larval lipophorin and the two adult forms are compared in Fig. 4. It can be seen that apolipophorin I and II are present in all forms, but that apolipophorin III is present only in the adult forms and is much more prominent in the low density (loaded) adult form. This shows that lipid loading is accompanied by association of several

TABLE I. Total fatty acid content of larval and adult (unloaded and lipid-loaded) lipophorins. Adult lipophorin samples were taken from the gradients graphed in figures 2 and 3, identified by fraction numbers below. Larval lipophorin was from a routine preparation (9). Protein determinations were done in duplicate, lipid determinations in triplicate. Mean \pm S.D.

Lipophorin	Lipid/Protein (μ moles fatty acid/mg protein)	
	-AKH	+AKH
Larval	1.41 \pm 0.10	----
Adult male		
Unloaded (fracts. 14-15)	2.89 \pm 0.23	3.61 \pm 0.19
Loaded (fracts. 7-9)	6.50 \pm 0.90*	5.98 \pm 0.02

*Lipid determinations fell below lowest triolein standards.

molecules of apoLp-III. It is likely that expansion of the particle diameter and reorganization of the surface layer requires the addition of apoLp-III to produce a maximally stabilized particle (16).

Table I shows the total lipid content of the three lipophorin forms. The ratio of lipid to protein increased from the larval form to the adult form, as might be expected from the decrease in density. The decrease in density of lipophorin after AKH stimulation corresponds to an approximate doubling in lipid content. These changes are actually more dramatic than the figures indicate, since apoLp-III is added to the particle simultaneously with lipid.

It is of interest that the locust hormone acts in such divergent insect species. O'Shea et al. (18) have recently reported two new peptide hormones from M. sexta corpora cardiaca that have lipid mobilizing activity in the locust. One or both of these are no doubt the M. sexta native hormones that promote lipid mobilization. It has also been reported that a peptide from crustacea, the shrimp red-pigment-concentrating hormone, which has homology with adipokinetic hormone (19) causes lipid mobilization in the locust (20). Thus, the adipokinetic hormones seem to represent a closely related group of peptides, widely distributed among the arthropods and serving analogous as well as diverse functions.

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