

ADIPOKINETIC HORMONE CAUSES FORMATION OF A LOW DENSITY LIPOPHORIN IN THE HOUSE CRICKET, *ACHETA DOMESTICUS*

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Abstract—In the house cricket, *Acheta domesticus*, injection of adipokinetic hormone induces the formation of a low density lipophorin via uptake of diacylglycerol from the fat body. Cricket low density lipophorin contains apolipophorin-III, which was purified and partially characterized. Cricket apolipophorin-III has a molecular weight of about 18,000, is not glycosylated, and has an isoelectric point of 4.8. Its amino acid composition is more similar to apolipophorin-III from *Locusta migratoria* than to that from *Manduca sexta*. The amino terminal sequence of cricket apolipophorin-III shows only limited homology to the amino terminal sequences of apolipophorin-III from *L. migratoria* and *M. sexta*.

Key Word Index: lipophorin, apolipophorin-III, cricket, *Acheta domesticus*, adipokinetic hormone

INTRODUCTION

The role of adipokinetic hormone (AKH) in lipid mobilization during flight has been well studied in *Locusta migratoria* and *Manduca sexta* (see Shapiro *et al.* 1988; Kanost *et al.*, 1990 for recent reviews). In these species AKH induces formation of diacylglycerol in the fat body which subsequently leaves the fat body and associates in hemolymph with high density lipophorin (HDLp) to form a low density lipophorin (LDLp). Concomitant with uptake of diacylglycerol, a small water-soluble apolipoprotein, apolipophorin-III (apoLp-III), binds to LDLp. ApoLp-III is thought to increase the lipid-carrying capacity of lipophorin by stabilizing the increment of lipid-water interface resulting from diacylglycerol uptake (Kawooya *et al.*, 1986; Wells *et al.*, 1987). LDLp is then carried by circulating hemolymph to flight muscle where fatty acids are released with reformation of HDLp and free apoLp-III.

It appears that some insect species which have lost, during evolution, the ability to fly have retained components of the flight-related lipid transport system. In the flightless grasshopper, *Barytettix psolus*, AKH does not mobilize lipids in spite of the presence of HDLp, apoLp-III and AKH (Ziegler *et al.*, 1988). It has been reported, however, that AKH causes lipid mobilization in the cricket, *Acheta domesticus*, which does not fly (Woodring *et al.*, 1989), although the authors did not demonstrate the formation of LDLp or the presence of apoLp-III. HDLp has been isolated from *A. domesticus* and does not contain apoLp-III (Ryan *et al.*, 1984). Since *A. domesticus* does not fly, yet responds to AKH with mobilization of lipid, we felt it worthwhile to investigate further its response to AKH, particularly the presence of apoLp-III and the formation of LDLp.

MATERIALS AND METHODS

Insects

Crickets were obtained from Fluker's Cricket Farm, Baton Rouge, La and were provided with water, dog food and potato slices in a 20 gal plastic container. Both male and female adult insects were used in these experiments with no detectable difference in the results.

Purification and characterization of apoLp-III

Crickets were bled by the flushing out method (Chino *et al.*, 1987) using a 50 mM Tris buffer pH 7.5 containing 150 mM NaCl, 10 mM glutathione and 1 mM diisopropyl-fluorophosphate. After low speed centrifugation to remove hemocytes, the hemolymph was heated in a boiling water bath for 5 min. Precipitated proteins were removed by centrifugation (5000 g, 10 min), the supernatant was desalted on a column of Sephadex G-25 (PD-10 column, Pharmacia) eluted with water and apoLp-III was purified by HPLC as described previously (Cole *et al.*, 1987). For amino acid analysis of apoLp-III, duplicate samples were hydrolyzed in 6 N HCl at 110°C *in vacuo* for 24 h and analyzed on a Beckman 7300 amino acid analyzer. NH₂-terminal sequencing was by automated Edman degradation as described by Cole *et al.* (1987). SDS-PAGE was carried out in 10% polyacrylamide gels (Laemmli, 1970). Isoelectric focusing was conducted in pH 3-9 gel (Phast system, Pharmacia). Carbohydrate was assayed by the phenol-sulfuric acid method (Ashwell, 1966).

Isolation and characterization of lipophorins

HDLp was prepared from hemolymph collected from resting insects as described above. LDLp was prepared from hemolymph collected 60 min after injection of 100 pmol of *Periplaneta* MI (Penninsula, Belmont, Calif.) (O'Shea *et al.*, 1984) in 10 µl of water. MI was chosen because it was the most similar peptide to *A. domesticus* AKH that was commercially available. HDLp and LDLp were isolated by density gradient centrifugation and analyzed for protein and lipid as previously described (Prasad *et al.*, 1986; Ryan *et al.*, 1986).

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Table 1. Properties of lipophorins from *Acheta domesticus*, *Manduca sexta* and *Locusta migratoria*. Composition is weight %; density in g/ml

	Protein	Lipid	PL*	DG*	HC*	Density
HDLp						
<i>A. domesticus</i> †	57.2	42.8	14.0	18.0	4.5	1.106
<i>M. sexta</i> ‡	48.5	51.5	14.0	25.0	3.5	1.076
<i>L. migratoria</i> §	59.0	41.0	14.8	13.4	8.7	1.12
LDLp						
<i>A. domesticus</i> †	47.8	52.2	8.9	36.6	3.5	1.061
<i>M. sexta</i>	37.8	62.2	7.1	46.9	2.3	1.03
<i>L. migratoria</i> ¶	53.7	46.3	10.9	26.1	6.4	1.065

*PL = phospholipid, DG = diacylglycerol, HC = hydrocarbon.

†Also contains triacylglycerol, sterol and free fatty acids in amounts less than 1.5%.

‡Prasad *et al.* (1966).

§Chino and Kitazawa (1981).

||Ryan *et al.* (1986).

¶Chino and Yazawa (1986).

RESULTS AND DISCUSSION

AKH induced formation of LDLp

To test whether AKH can cause formation of LDLp in *A. domesticus*, 100 pmol of AKH (*Periplaneta* MI) was injected into adult crickets and after 1 h the animals were bled, and lipophorin was isolated by density gradient centrifugation and characterized with respect to density, protein content and lipid content (Table 1). In untreated crickets HDLp was present, with an average density of 1.106 g/ml, similar to the density previously determined (Ryan *et al.*, 1984). After injection of AKH, lipophorin was in the form of LDLp with a density of 1.061 g/ml. The lipid content of lipophorin increased from 42.8 to 52.2%, due to elevated amounts of diacylglycerol in LDLp. When properties of cricket HDLp and LDLp are compared to those of *M. sexta* and *L. migratoria* (Table 1), it can be seen that the cricket HDLp is similar to locust HDLp, but that cricket LDLp has properties intermediate between locust and hornworm LDLp.

SDS-PAGE analysis of HDLp and LDLp from *A. domesticus* shows that HDLp contains only apolipoprotein-I (apoLp-I) and apolipoprotein-II (apoLp-II), whereas LDLp contains apoLp-III in addition to apoLp-I and -II (Fig. 1). Thus, the adipokinetic response of *A. domesticus* involves mobilization of lipid, resulting in formation of LDLp with associated apoLp-III, very similar to the re-

sponses of *L. migratoria* and *M. sexta*, which use lipid to fuel flight.

Isolation and characterization of apoLp-III

An understanding of the structure and physical properties of apoLp-III seems essential for understanding how lipophorin functions as a lipid shuttle when LDLp is formed in response to AKH. ApoLp-III from *M. sexta* and *L. migratoria* has been extensively characterized (Kawooya *et al.*, 1986; Wells *et al.*, 1987; Cole *et al.*, 1987; Kanost *et al.*, 1988), including determination of amino acid sequences deduced from cDNA sequences. The *L. migratoria* and *M. sexta* apoLp-III amino acid sequences are only 29% identical, and although it is suspected that they may form amphipathic helices, it is difficult from comparison of the two sequences to predict which parts of the proteins are functionally most important. Characterization of apoLp-III from other species may yield information on common structures which may be essential for function. Thus, we have isolated and characterized apoLp-III from *A. domesticus* as a first step toward complete sequence determination through cDNA cloning.

ApoLp-III was isolated by a method similar to that used previously for *L. migratoria* apoLp-III (Kanost *et al.*, 1988). The apoLp-III protein is very stable to

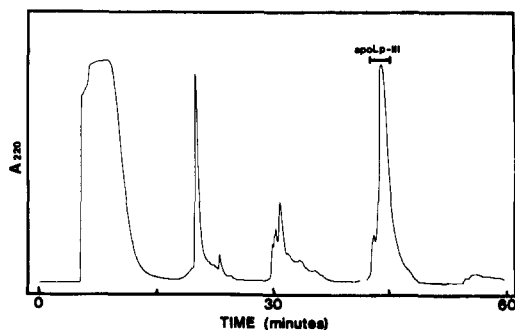


Fig. 2. Isolation of apoLp-III by reversed phase HPLC. Five mg of heat stable proteins from *A. domesticus* hemolymph were applied to a C8 column (Vydac, 10 × 250 mm) and eluted with an acetonitrile gradient as described by Cole *et al.* (1987).

Table 2. Amino acid composition of apolipoprotein-III from *Acheta domesticus*, *Manduca sexta* (Cole *et al.*, 1987) and *Locusta migratoria* (Kanost *et al.*, 1988). Data are in residues per molecule

Amino acid	<i>A. domesticus</i>	<i>L. migratoria</i>	<i>M. sexta</i>
Asx	15	18	19
Thr	12	11	7
Ser	8	11	12
Glx	37	31	34
Pro	4	7	3
Gly	5	4	5
Ala	35	29	24
Val	10	9	9
Met	0	0	2
Ile	3	5	2
Leu	11	15	11
Tyr	0	0	1
Phe	6	2	8
His	4	7	4
Lys	4	7	24
Arg	7	3	2
Trp	ND*	2	0
Cys	0	0	0

*Not determined.

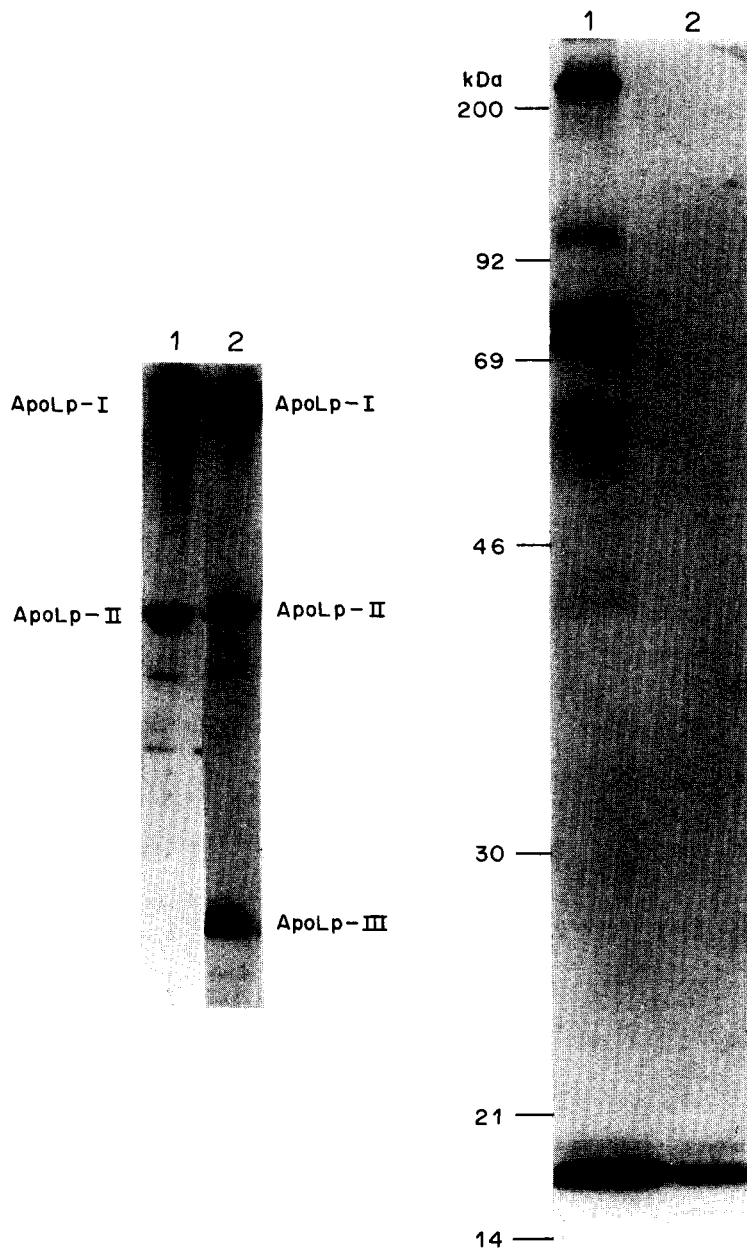


Fig. 1

Fig. 3

Fig. 1. SDS-PAGE analysis of apoprotein composition of *A. domesticus* lipophorins. Lane 1: HDLp isolated from untreated insects. Lane 2: LDLp from insects 1 h after injection of AKH. Each lane represents 20 μ l of a KBr density gradient fraction.

Fig. 3. SDS-PAGE analysis of purified apoLp-III. Lane 1: hemolymph (1 μ l) from adult male *A. domesticus*. Lane 2: purified apoLp-III (10 μ g). The position of molecular weight markers is shown on the left.

Table 3. Comparison of N-terminal amino acid sequence of apoLp-III from *Acheta domesticus*, *Manduca sexta* (Cole *et al.*, 1987) and *Locusta migratoria* (Kanost *et al.*, 1988)

<i>L. migratoria</i>	DAAGHVNI AEA VQQLNHTIV...
	: : :
<i>A. domesticus</i>	DAGTTGADFN S L F E A A Q M A F...
	: : : : : : :
<i>M. sexta</i>	DAPAGGNA F E E M E K H A K E F Q...

heat, so heat treatment provides a separation from most higher molecular weight hemolymph proteins. Reverse phase HPLC separation of the heat stable proteins (Fig. 2) resulted in a preparation of apoLp-III which appeared homogeneous when examined by SDS-PAGE (Fig. 3). The purified protein has a molecular weight of approx. 18,000 and an isoelectric point of 4.8. In contrast to the *L. migratoria* apoLp-III (Chino *et al.*, 1986; Ryan *et al.*, 1990), the cricket apoLp-III contains no detectable carbohydrate (<1%). The amino acid composition of cricket apoLp-III is compared to apoLp-III from *L. migratoria* and *M. sexta* in Table 2. The cricket apoLp-III is more similar to the *L. migratoria* apoLp-III, especially in its low content of lysine. This probably accounts for the lower isoelectric points of the cricket (pI = 4.8) and locust (pI = 5.0) proteins when compared with *M. sexta* apoLp-III (pI = 6.1).

A partial NH₂-terminal sequence of the cricket apoLp-III is compared to that of *L. migratoria* and *M. sexta* apoLp-III in Table 3. The sequence similarity between the three proteins is not striking. All three proteins have Asp-Ala at the NH₂-terminus, with 5/20 identical residues between *A. domesticus* and *M. sexta* and only 2/20 identical between *A. domesticus* and *L. migratoria*. It is probable that conservation of patterns of amino acids with similar properties may have resulted in evolution of functional apoLp-III with little sequence identity. We hope that by determining the sequence of a larger number of apoLp-III molecules these patterns will become apparent. Specific antibodies to cricket apoLp-III and a cricket fat body cDNA library have been prepared (Strobel, Hanneman and Kanost, unpublished), and cDNA sequences for apoLp-III from *A. domesticus* and other species should be useful in solving this problem.

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