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Anopheles gambiae lipophorin: Characterization and role in lipid transport to developing oocyte

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Abstract

Lipid transport in arthropods is achieved by highly specialized lipoproteins, which resemble those described in vertebrate blood. Here, we describe purification and characterization of the lipid–apolipoprotein complex, lipophorin (Lp), in the malaria vector mosquito *Anopheles gambiae*. We also describe the Lp-mediated lipid transfer to developing eggs and the distribution of the imported lipid in developing embryos. The density of the Lp complex was 1.135 g/ml with an apparent molecular weight of 630 kDa. It is composed of two major polypeptides, apoLp I (260 kDa) and apoLp II (74 kDa) and composed of 50% protein, 48% lipid and 2% carbohydrate (w/w). Hydrocarbon, cholesterol, phosphatidyl choline, phosphatidyl ethanolamine, cholesteryl ester and diacylglyceride were the major Lp-associated lipids. Using fluorescently tagged lipids, we observed patterns that suggest that in live developing oocytes, the Lp was taken up by a receptor-mediated endocytic process. Such process was blocked at low temperature and in the presence of excess unlabeled Lp, but not by bovine serum albumin. Imported Lp was segregated in the spherical yolk bodies (mean size 1.8 μm) and distributed evenly in the cortex of the oocyte. In embryonic larvae, before hatching, a portion of the fatty acid in vesicles was found evenly distributed along the body, whereas portion of phospholipids was accumulated in the intestine.

Keywords: Lipoprotein; Mosquito; Oogenesis; Lipid transport; Insect; Disease vector; Malaria

1. Introduction

Mosquitoes are vectors of many human diseases including malaria, yellow fever, filariasis, dengue and West Nile Virus. Attempts to eradicate mosquitoes by insecticide have failed, because of the emergence of insecticide-resistant mosquitoes. An effective strategy to control mosquito population is to interfere with egg laying and reducing larval density. Rapid increase of mosquito population in optimal seasons is due to the laying of several hundreds of eggs in a few days. In most vector mosquitoes, egg development requires vertebrate blood components. Therefore, understanding mosquito egg development and the role of host blood components in the process may identify novel ways to control the rapid proliferation of these potentially deadly disease-carriers.

It is known that lipids are carried as lipoprotein complexes and delivered to target tissues. However, it is not clear how different egg-laying animals transport nutrients to developing eggs and store nutrients for neonates to use until it ingests its first meal. Lipid compositions in eggs and neonates of different egg-laying and live-bearing lizards was found to be remarkably different (Speake and Thompson, 2000; Thompson et al., 2000). In the liver of the neonate chick, lipid composition showed an increase in cholesteryl ester and a decrease in triglycerides after birth (Noble and Ogunyemi, 1989; Noble and Cocchi, 1990). Lipid transport in vertebrates

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is orchestrated by chylomcron, very-low-density (VLDL), low-density (LDL), intermediate-density (IDL) and high-density (HDL) lipoproteins. Whereas in insects, lipid transport occurs via lipophorin (Lp), a class of HDL (Chino et al., 1981; Kawooya and Law, 1988; Arrese et al., 2001).

Lps are complexes of lipids and apoproteins that carry and distribute different classes of lipids through insect hemolymph to various tissues (Chino et al., 1981; Shapiro et al., 1988; Atella et al., 1992). The role of Lp as the major lipid vehicle for lipid transport to the oocytes was shown in *Philosamia cynthia* (Chino et al., 1977) and *Manduca sexta* (Kawooya et al., 1988). Most insect Lps contain two apolipoproteins—apolipoprotein I (~250 kDa) and apolipoprotein II (~80 kDa), which comprise about 60% of the mass of the lipid/protein complex (Chino, 1985). A third apoprotein (apolipoprotein III) of molecular mass ~17 kDa is present in adults of some species (Chino et al., 1986). Also, it is well demonstrated that the molar ratio of the apoproteins is a function of the insect physiological state (Shapiro and Law, 1983; Chino, 1985; Kawooya et al., 1986). Like the apoproteins, lipid composition also varies depending on the insect and its physiological state. Generally, the major lipids associated with lipophorin are diacylglycerides, cholesterol, hydrocarbons and phospholipids (Thomas and Gilbert, 1968; Chino et al., 1969; Peled and Tietz, 1973; Gondim et al., 1989).

Insect Lps may function as reusable shuttles for lipid transport (Blacklock and Ryan, 1994; Soulages et al., 1994; Arrese et al., 2001; van der Horst et al., 2002). For example, Lp complexes transfer diacylglycerols to flight muscles and the protein can be recycled back to the hemolymph. Also in *M. sexta*, low-density Lps (LDLp) first unloads its lipids to the fat body. Then the apolipoprotein is recycled back to the hemolymph (Kawooya et al., 1988). During oogenesis, a rapid accumulation of proteins and lipids occur in the oocytes, hence facilitating the eggs to mature in a relatively short time. This process is complex and the involvement of several tissues coordinated by hormones has been demonstrated (Engelmann, 1979). High-density Lp (HDLp) also transfers lipids without recycling of apoproteins (Kawooya et al., 1988). The presence of a Lp receptor *Aedes aegypti* suggested that in this mosquito, the internalization of Lp occurs via endocytosis (Cheon et al., 2001). The internalized Lp acts as yolk protein precursor (Kulakosky and Telfer, 1990; Sun et al., 2000). Despite the evidence suggesting that Lp is internalized in a developing oocyte by endocytosis (Van Antwerpen et al., 1993), few clear observations of endocytic vesicles in oocytes have been demonstrated. Recently, Van Hoof et al. (2005) examined lipoprotein and transferrin trafficking in insect cells. Using a lipoprotein-specific antibody with Lp receptor gene transfected cultured cells, endocytic vesicles were observed by these authors. However, studies of endocytosis have proven to be difficult with fat body due to the fragility and irregular composition of the tissue (Locke, 1998).

Here, we describe the purification and characterization of malaria vector mosquito *Anopheles gambiae* Lp and the use of fluorescently labeled Lp to examine the process of lipid transfer in live developing oocytes. The results showed that lipophorin is taken up via endocytosis by mosquito eggs. We also examined the distribution of imported lipids in maturing eggs and in developing embryos. This showed that imported endocytic vesicles fuse to form the yolk bodies until they reach to a certain size. Imported fatty acids and phospholipids remained stored in yolk bodies during egg development. However, some of these lipids apparently remain unused during egg and embryonic development, and are distributed in different tissues in newly hatched larvae.

2. Materials and methods

2.1. Mosquito rearing

The *An. gambiae* (G3 strain) colony was maintained under standard insectary conditions (27 °C and 80% relative humidity, photoperiodism: 12L–12D) and fed on a diluted Karo syrup (CPC International Inc., Englewood Cliffs, NJ) saturated cotton ball. For blood feeding, 6–8 days after emergence from pupae, adult female mosquitoes were starved overnight and fed ad libitum on 4- to 5-week-old White Leghorn chickens. Fed mosquitoes were separated and kept in the insectary until used.

2.2. Lp purification and composition analysis

An. gambiae Lp was purified as described earlier with minor modifications (Atella and Shahabuddin, 2002). Five to 10 days after emergence from pupae, sugar-fed mosquitoes (4 g) were snap frozen under liquid nitrogen and ground to a powdered form with a ceramic grinder (Fisher Scientific, Pittsburgh, PA). The powder was immediately transferred to the homogenization solution (10 mM phosphate, 0.15 M NaCl, pH 7.4) containing glutathione (20 mM), EDTA (5 mM), PMSF (2 mM), antipain (0.5 μg/μl), pepstatin (5 μM) and leupeptin (0.5 μg/μl) on an ice bath. The homogenate was centrifuged at 100,000 × *g*, for 30 min at 4 °C. Solid KBr was added to the supernatant to a final concentration of 0.4 g/ml and the mixture was again centrifuged at 125,000 × *g* in a Beckman ultracentrifuge (Optima L-90 ultracentrifuge, Beckman Coulter, Palo Alto, CA) with a fixed angle Beckman rotor Ti50.2 at 4 °C for 20 h. Supernatant was collected from the top as fractions. Presence of Lp in each fraction was checked using SDS polyacrylamide gel. Fractions with typical banding pattern of Lp were pooled and extensively dialyzed against PBS. Samples were then concentrated using a vacuum dryer (speed vac, Savant) and stored under liquid nitrogen.

Protein concentration was estimated using micro BCA Kit (Pierce, Rockford, IL) in the presence of 0.5% SDS, using bovine serum albumin (BSA) as standard. Each