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The process of lipid storage in insect oocytes: The involvement of β-chain of ATP synthase in lipophorin-mediated lipid transfer in the chagas' disease vector *Panstrongylus megistus* (Hemiptera: Reduviidae)

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ABSTRACT

Lipophorin is the main lipoprotein in the hemolymph of insects. During vitellogenesis, lipophorin delivers its hydrophobic cargo to developing oocytes by its binding to non-endocytic receptors at the plasma membrane of the cells. In some species however, lipophorin may also be internalized to some extent, thus maximizing the storage of lipid resources in growing oocytes. The ectopic β chain of ATP synthase (β-ATPase) was recently described as a putative non-endocytic lipophorin receptor in the anterior midgut of the hematophagous insect *Panstrongylus megistus*. In the present work, females of this species at the vitellogenic stage of the reproductive cycle were employed to investigate the role of β-ATPase in the transfer of lipids to the ovarian tissue. Subcellular fractionation and western blot revealed the presence of β-ATPase in the microsomal membranes of the ovarian tissue, suggesting its localization in the plasma membrane. Immunofluorescence assays showed partial co-localization of β-ATPase and lipophorin in the membrane of oocytes as well as in the basal domain of the follicular epithelial cells. Ligand blotting and co-immunoprecipitation approaches confirmed the interaction between lipophorin and β-ATPase. *In vivo* experiments with an anti-β-ATPase antibody injected to block such an interaction demonstrated that the antibody significantly impaired the transfer of fatty acids from lipophorin to the oocyte. However, the endocytic pathway of lipophorin was not affected. On the other hand, partial inhibition of ATP synthase activity did not modify the transfer of lipids from lipophorin to oocytes. When the assays were performed at 4 °C to diminish endocytosis, the results showed that the antibody interfered with lipophorin binding to the oocyte plasma membrane as well as with the transfer of fatty acids from the lipoprotein to the oocyte. The findings strongly support that β-ATPase plays a role as a docking lipophorin receptor at the ovary of *P. megistus*, similarly to its function in the midgut of such a vector. In addition, the role of β-ATPase as a docking receptor seems to be independent of the enzymatic ATP synthase activity.

Abbreviations: β-ATPase, β-chain of the ATP synthase complex; Bodipy FL C16, 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-hexadecanoic acid; Cameo2, C locus associated membrane protein homologous to a mammalian HDL receptor-2; CD36, cluster of differentiation 36; DAG, diacylglycerol; DAGTP, diacylglycerol transport protein; DIC, differential interference contrast; DiI, 1,10-dioctadecyl-3,3,30,30-tetramethylindocarbocyanine; DTSSP, 3,3′-dithiobis(sulfosuccinimidyl propionate); HDL, high-density lipoprotein; JHBP, juvenile hormone binding protein; LDLR, low-density lipoprotein receptor; Lp, lipophorin; LpR, liphoporin receptor; LPL, lipoprotein lipase; LTP, lipid transfer particle; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SCRB15, scavenger receptor class B member 1 like protein 15.

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1. Introduction

Lipophorin is the main lipid carrier in the hemolymph of insects. Under most physiological conditions, lipophorin is a high-density lipoprotein and contains two structural apolipoproteins, apolipophorin I and II (Beenakkers et al., 1985; Canavoso et al., 2001; Van der Horst et al., 2009). Diacylglycerol is the major lipid transported by lipophorin but depending on the insect species and their developmental stage this lipoprotein can also carry other hydrophobic molecules such as fatty acids, hydrocarbons, sterols and phospholipids (Chino et al., 1969; Canavoso et al., 2001).

Lipophorin interacts with specific binding sites in the plasma membrane of the target cells and lipids are transferred mainly without endocytosis. Once the loading/unloading process is completed, the lipoprotein returns to the circulation for subsequent cycles of lipid mobilization (Chino and Gilbert, 1964; Chino and Downer, 1982; Arrese et al., 2001). In addition to this main pathway for lipid delivery, lipophorin can be either endocytosed by the oocytes as reported in several insect species (Kawooya and Law, 1988; Ziegler and Van Antwerpen, 2006) or endocytosed and resecreted, as in the fat body of *Locusta migratoria* (Van Hoof et al., 2005). The endocytic pathway is mediated by the lipophorin receptor (LpR) belonging to the Low-Density Lipoprotein Receptor (LDLR) family, which was first described in *L. migratoria* (Dantuma et al., 1999). Even though the presence of LpRs was reported in various insects, their physiological relevance for the transfer of neutral lipids to tissues remains elusive (Tufail and Takeda, 2009).

Other lipophorin receptor candidates include *Drosophila melanogaster* Dally and Dally-like, two membrane-associated heparan sulfate proteoglycans with lipophorin-binding capacity, although they appear to be more involved in cell signaling than in lipid transfer (Eugster et al., 2007). Cameo2 and SCRB15, two transmembrane proteins expressed in the silk gland of *Bombyx mori* function as non-endocytic lipophorin receptors that facilitate the selective uptake of carotenoids. However, the role of these CD36 family members in the general transfer of neutral lipids is still unknown (Sakudoh et al., 2013).

The multiprotein F_1F_o-ATP synthase complex was originally thought to be located exclusively in the inner membrane of mitochondria. This complex employs a transmembrane proton motive force to drive the chemical synthesis of ATP from ADP and inorganic phosphate (Walker, 2013). Notwithstanding, several lines of evidence confirmed the presence of ATP synthase in the plasma membrane of different mammalian cell types, mediating a variety of biological processes such as the metabolism of high-density lipoproteins, endothelial cell proliferation and antitumor activity (Vantourout et al., 2010). This surface or ectopic ATP synthase, as it was termed to distinguish it from its mitochondrial counterpart, was also described as a protein which binds different ligands in arthropods (Giot et al., 2003; Zalewska et al., 2009). Very recently, we have proposed that β-chain of the ATP synthase complex (β-ATPase) plays a role as non-endocytic lipophorin receptor in the anterior midgut of the triatomine *Panstrongylus megistus*, an important vector of Chagas' disease (Fruttero et al., 2014).

During oogenesis, lipids must be stored in growing oocytes as they are the main source of energy to sustain the development of the embryos. However, in the context of insect reproductive physiology, the process of lipid transfer to oocytes is still not completely understood (Ziegler and Van Antwerpen, 2006). Previous studies in vitellogenic females of the triatomines *P. megistus* and *Dipetalogaster maxima* allowed us to demonstrate that the storage of lipid resources by developing oocytes involves the convergence of non-endocytic and endocytic pathways of lipophorin to maximize the delivery of its lipid cargo (Fruttero et al., 2011; Leyria et al., 2014).

P. megistus belongs to the subfamily Triatominae, a group of hematophagous insects with relevance in public health as they are vectors of Chagas' disease (Schofield et al., 2006). The female of *P. megistus* takes large blood meals, rich in lipids and proteins. The blood meal triggers vitellogenesis, which in *P. megistus* can be broadly divided into early, mid and late vitellogenesis; the latter coincides with the oviposition period. During vitellogenesis, terminal oocytes remarkably enlarge due to internalization of yolk protein precursors and uptake of circulating lipids via lipophorin-mediated lipid transfer (Fruttero et al., 2011). In this work, we analyzed the role of the β-ATPase in the process of lipophorin-mediated lipid transfer to the ovarian tissue. Immunofluorescence, ligand blotting and co-immunoprecipitation approaches supported both, the binding and interaction between lipophorin and β-ATPase. Different *in vivo* assays directed to block such an interaction revealed the importance of β-ATPase in the process of lipid accumulation by developing oocytes. Finally, it was observed that blocking β-ATPase did not significantly impair lipophorin endocytosis.

2. Materials and methods

2.1. Chemicals

4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-hexadecanoic acid (Bodipy FL C16) and goat anti-rabbit IgG labeled with Alexa 568 antibody (Molecular Probes, Eugene, OR, USA), rabbit anti-ATP5B/β-chain of ATP synthase of human origin (catalog code sc-33618) and rabbit anti-cytochrome c of equine origin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA); Sephadex G-25 PD-10 columns (GE Healthcare, Little Chalfont, UK); Tissue-Tek embedding medium Optimal Cutting Temperature (OCT) (Miles, Elkhart, IN, USA); Centricon devices (Millipore, Bedford, MA, USA); Enliten bioluminescence detection kit (Promega, Madison, WI, USA); Fluorsave (Calbiochem, Darmstadt, Germany); 3,3′-dithiobis(sulfosuccinimidyl propionate) (DTSSP) (Thermo Scientific, Rockford, IL, USA) and Color Prestained Protein Standard (New England Biolabs Inc, Ipswich, MA, USA) were from the indicated commercial sources. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); Bovine Serum Albumin (BSA); Dimethyl sulfoxide (DMSO); dimethylpimelimidate (DMP); 1,10-dioctadecyl-3,3,30,30-tetramethylindocarbocyanine (DiI); fluorescein isothiocyanate (FITC); Fetal Bovine Serum (FBS); oligomycin and all the other chemicals were from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Insects

Experiments were carried out with insects taken from a colony of *P. megistus*, maintained at 28 °C, 70% relative humidity, 8:16 h light:dark photoperiod. Insects were fed on hen blood (Canavoso and Rubiolo, 1995), according to the recommendations of the National Institute of Parasitology (Health Ministry, Argentina) (Núñez and Segura, 1987). Standardized conditions of insect rearing were previously described (Fruttero et al., 2011). Briefly, fifth-instar females were separated from males before feeding. Newly emerged females were segregated individually and placed together with two recently fed males. Mating was checked by observation of the spermatophore. Mated females were maintained in individual containers until they were able to feed a blood meal (day 7 post-ecdysis). Experimental approaches were performed by sampling hemolymph and ovaries from females at early vitellogenesis, which in *P. megistus* correspond to days 3–4 after blood feeding (Fruttero et al., 2011).