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Energy sources from the eggs of the wolf spider *Schizocosa malitiosa*: Isolation and characterization of lipovitellins

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ABSTRACT

In oviparous species, proteins and lipids found in the vitellus form the lipoproteins called lipovitellins that are the major source of energy for the development, growth, and survival of the embryo. The energy resources provided by the lipovitellins have not yet been investigated in the Order Araneae. Using the wolf spider *Schizocosa malitiosa* (Lycosidae) as an experimental model, we identified and characterized the lipovitellins present in the cytosol, focusing on the energetic contribution of those lipoprotein particles in the vitellus. Two lipovitellins (LV) named SmLV1 and SmLV2 were isolated. SmLV1 is a high-density lipoprotein with 67% lipid and 3.6% carbohydrate, and SmLV2 is a very high-density lipoprotein with 9% lipid and 8.8% carbohydrate. Through electrophoresis in native conditions we observed that SmLV1 has a molecular mass of 559 kDa composed of three apolipoproteins of 116, 87, and 42 kDa, respectively. SmLV2 comprised several proteins composed of different proportions of the same subunits (135, 126, 109, and 70 kDa). The principal lipids of these lipovitellins are sphingomyelin + lysophosphatidylcholine, esterified sterols, and phosphatidylcholine. Lipovitellin-free cytosol contains abundant phospatidylcholine and triacylglyceride related to the *yolk nuclei* (the vitellus organizing center). The principal fatty acids of SmLV1 and SmLV2 are 18:2 n−6, 18:1 n−9, and 16:0. Spectrophotometry detected no pigments in either the lipovitellins or the cytosol. The egg caloric content was 92 cal/g, at proportions of 59.8% protein, 20.1% carbohydrate, and 19.9% lipid. SmLV1 and SmLV2 provided 19.5% and 17.1% of the calories, respectively. Both lipovitellins contribute mainly with proteins (15.8–18%), with the input of carbohydrates and lipids being lower than 1.3%.

1. Introduction

In oviparous species, the main content of nutrients is found in the vitellus or yolk, it being essential for embryonic development. In general the vitellus in invertebrates is composed of lipids, proteins, and carbohydrates, and in many instances, it also contains pigments. Most eggs store nutrients in the yolk in the form of a complex lipoprotein called lipovitellin (LV), or as lipid droplets scattered throughout the cytoplasm. Vitellogenesis, the process of vitellus accumulation, in most invertebrates involves a massive synthesis of vitellogenin (the lipoprotein exclusive to females in the reproductive state) along with the latter deposition in the oocyte in the form of vitellins (majority protein of eggs or LVs, which are lipoproteins present in the vitellus Wallace et al., 1967).

Although terrestrial-arthropod vitellins have been well characterized in insects (Dhadialla and Raikhel, 1990; Chino, 1997; Salerno et al., 2002; Tufail and Takeda, 2008) as well as in aquatic arthopods such as crustaceans (Lubzens et al., 1997; Kawazoe et al., 2000; Chen et al., 2004; Garcia et al., 2006; Walker et al., 2006), the relevant data for arachnids are scarce with the exception of Acari, with vitellins in that group having been reported in several publications. In that order, LVs have been characterized in several species of ticks, containing either vitellins or vitellogenins; the latter are complex hemoglycolipophosphoproteins, compounds of structures assumed to be related mainly to the hematophagia of those ticks. Their lipid and carbohydrate composition is quite similar to that of the vitellins in insects (Tatchell, 1971; Boctor and Kamel, 1976; Chinzei et al., 1983; Dhadialla, 1986; Shanbaky et al., 1990; Schriefer, 1991; Rosell and Coons, 1992; James and Oliver, 1997; Boldbaatar et al., 2010).

The vitelline body—a typical structure studied and reported only in the oocytes of many spider families (von Wittich, 1845)—was found through microscopy to be surrounded by concentric lamellae corresponding to the endoplasmic reticulum (André and Rouiller, 1957; Sotelo and Trujillo-Cenóz, 1957). With respect to function, analyzing the structure through microscopy and based on morphology, Osaki (1972) determined that the vitellin body was most likely an organizing center for the yolk.

In the Order Araneae (spiders) two LVs have been described in *Polybetes pythagoricus* (Sparassidae), named LV1 and LV2. LV1 has

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49% lipids and a density corresponding to a high-density lipoprotein (HDL) and LV2 has a much lower lipid content (9.7%) and the density of a very high density lipoprotein (VHDL) (Laino et al., 2011). The main neutral lipid found was esterified cholesterol at 16 and 24% in LV1 and LV2, respectively; though triacylglycerides (TAG) were also present at 8 and 9.5% along with diacylglycerides at 3 and 4.3%, respectively. With respect to structural lipids, phospholipids were the dominant class at 57 and 47% in LV1 and LV2, respectively. The major fatty acid was 18:1 n−9 in both LVs. Since these particles were not related to hemolymphatic lipoproteins, we assume that the two lipoproteins are reproduction specific (i.e., vitellogenin/LV). Moreover, a putative apovitellin has been described in the ovary and eggs of the spider *Tegenaria atrica* (Agelenidae) (Pourié and Trabalon, 2003).

LVs also have a significant relevance to other lipoproteins. For example, Baker (1988) determined that the vitellogenin of certain invertebrates contains domains homologous to the apoB-100 of the human LDL and human lipase. Moreover, in a comparative genomic and phylogenetic analysis in metazoa, Hayward et al. (2010) recently determined that the genes encoding the LLTPs (i.e., proteins related to vitellogenin/LV) arose significantly earlier in evolution than the arachnids and are in addition more widespread than previously proposed, with those proteins being present in numerous additional bilaterian and nonbilaterian lineages.

In the present work, using the wolf spider *Schizocosa malitiosa* as an experimental model mainly due to its known embryo development lasting about 30 days (Costa and Capocasale, 1985), we identified and characterized the LVs present in the egg cytosol at an early stage of development, with an emphasis on the energetic contribution of those lipoprotein particles to the egg vitellin. The present study constitutes the first investigation of this nature performed in the widespread Order Araneae.

2. Materials and methods

2.1. Animal and lipovitellin isolation

A total of 30 subadult females of wolf spider were caught in Marinda, Canelones (Uruguay) and kept with food (mealworm larvae) in glass jars until they reach adulthood. Afterward, they mated with adult males in open arenas and were raised until spawning. Eggs from 20 egg-sacs (0.33 g/egg-sac) were collected for 3–7 days after laying (due to the great content of vitellus typical of early stages), homogenized in two pools in 3 mL of 50 mM potassium phosphate buffer, pH 7.4 (Buffer A) supplemented with 0.1% (v/v) protease-inhibitor cocktail (Sigma-Aldrich Chemicals, St. Louis, MO, USA), and sequentially centrifuged at 10,000 *g* for 20 min, and then at 100,000 *g* for 60 min to obtain the cytosol. This final cytosolic fraction, upon thawing, was used for the isolation of LVs by density-gradient ultracentrifugation as follows. All assays were performed in triplicate and with independent treatments.

Aliquots of cytosol were overlayered onto a NaBr solution (density 1.28 g/mL) containing 0.01% (w/v) sodium azide and centrifuged at 178,000 *g* and 10 °C for 24 h in a Beckman L8 70 M centrifuge, with a SW 60 Ti rotor. Saline solution of the same density as that of samples was centrifuged in parallel to check the densities of each fraction and thus verify the correctness of the gradient formation. The total volume of each tube was sampled from top to bottom in 0.2 mL aliquots and the protein content of each fraction monitored spectrophotometrically at 280 nm. The zone in the gradient containing the LVs was separated as a whole fraction. The LV-containing samples were dialyzed in Buffer A. The total protein concentration of LV was measured colorimetrically by the method of Lowry et al. (1951). Measurements obtained in the cytosol without LVs were obtained deducting the values of LVs to those of cytosol.

2.2. Electron microscopy

Samples of LV were dialyzed in Buffer A and negatively stained with 2% (w/v) phosphotungstate for examination by electron microscopy (Forte and Nordhausen, 1986). Preparations were made at least in triplicate, viewed on a JEOL 100S electron microscope (JEOL Co., Japan) at 100 kV, and photographed at a final magnification of 120,000×. Approximately 100 free standing lipoprotein particles were measured in multiple photographs taken from different areas of the grid. An additional photograph with the buffer alone was taken as a negative control to confirm the absence of artifacts.

2.3. Gel electrophoresis

Apolipoproteins corresponding to LVs and to the other cytosolic proteins were analyzed by polyacrylamide gel electrophoresis. Separations were performed in 4–23% native and sodium dodecylsulfate (SDS) gels (Laemmli, 1970). The bands obtained under native conditions for one of the LVs (SmLV2) were purified from the gels by electroelution and then run on a 4–23% SDS gel. Molecular masses were calculated as previously described (Garín et al., 1996). Gels were either stained with Coomassie Brilliant Blue R-250 (Sigma-Aldrich Chemical Co.) or silver-stained.

2.4. Lipids and fatty acid characterization

Lipids were extracted following the method of Folch et al. (1957). Quantitative determination of lipid classes was performed by thin layer chromatography coupled to a flame ionization detector in an Iatroscan apparatus model TH-10 (Iatron Laboratories, Tokyo, Japan), after separation on Chromarods type S-III (Ackman et al., 1990; Lavarías et al., 2005). Lipid classes were quantified with monoacylglycerol as an internal standard. The total lipids were determined by gravimetry (Cunningham and Pollero, 1996).

Fatty acid methyl esters from LV total lipids were prepared with BF_3-MeHO according to the method of Morrison and Smith (1992). The analysis was performed by gas–liquid chromatography in a HP-6890 capillary chromatograph (Hewlett Packard, Palo Alto, CA, USA) on an Omegawax 250 30 m × 0.25 mm fused silica column with a 0.25 μm phase (Supelco, Bellefonte, CA, USA). The column temperature was programmed for a linear increase of 3 °C per min from 175 to 230 °C. Peaks were identified by comparing their retention times with those of a mixture of standard methyl esters.

2.5. Carbohydrate determination

Total hexose content of LVs was determined by the anthrone/sulfuric method with D-glucose as the standard (Sigma-Aldrich). The samples were treated with an anthrone solution in concentrated H_2SO_4. After 15 min at 90 °C the absorbance was read at 620 nm (Dubois et al., 1956).

2.6. Absorbance spectrum of LVs and cytosol

The absorbance spectum of LVs and cytosol was measured from 200 to 550 nm in an Agilent 8453 ultraviolet spectrophotometer. For the purpose of comparison it was also measured the absorbance spectrum of the VHDL of the spider *P. pythagoricus* (a hemocyanin-containing lipoprotein) along with ovorubin, a protein from the snail *Pomacea canaliculata* with carotenoid pigment.

2.7. Energetic equivalent of proteins, lipids, and carbohydrates in LVs and cytosol

In the present work, to estimate calories provided by different molecules we used the conversion factor described by Beningher