Comparative Biochemistry and Physiology, Part B 140 (2005) 381–386

CBP

www.elsevier.com/locate/cbpb

Characterization of lipovitellin in eggs of the polychaete *Neanthes arenaceodentata*

Richard F. Lee[a,*], Anna Walker[b], Donald J. Reish[c]

[a]*Department of Marine Biochemistry, Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, GA 31411, USA*
[b]*Department of Pathology, Mercer University School of Medicine, Macon, GA 31207, USA*
[c]*Department of Biology, California State University, Long Beach, CA 90840, USA*

Received 3 May 2004; received in revised form 31 October 2004; accepted 1 November 2004

Abstract

The ooplasm of mature oocytes of the polychaete *Neanthes arenaceodentata* is characteristically filled with yolk platelets. A major component of these structures is lipovitellin, which provides energy and materials required by newly hatched larvae. The lipovitellin isolated and purified from the fertilized eggs of this polychaete was a high-density lipoprotein composed of protein (57%), lipid (42%) and carbohydrate (1%). The lipid component included phospholipids (92% of lipid), triacylglycerol (3% of lipid) and cholesterol (3% of lipid), while sodium dodecyl sulfate-gel electrophoresis showed the major protein component was a 120-kDa peptide. Microscopically, mature oocytes were present in the coelom along with phagocytic eleocytes. The presence of muscle fragments and oil droplets in eleocytes suggests that eleocytes play an important role in providing the protein and lipid needed for the assembly of lipovitellin in the oocytes.

Keywords: Polychaete; Lipovitellin; Reproduction; Eleocytes; Eggs; *Neanthes*; Oocytes; Lipid; Lipoprotein

1. Introduction

The process of vitellogenesis includes the accumulation of yolk in the developing oocytes and has been described in a number of polychaete species. Depending on the species, yolk synthesis has been found to occur both within and outside of the ovary (Eckelbarger, 1983; Eckelbarger and Grassle, 1982; Fischer, 1979; Fischer and Hoeger, 1993; Fischer and Rabien, 1986; Fischer et al., 1996; Porchet et al., 1989; Schechtman, 1955). The major component of the egg yolk of most marine invertebrates is lipovitellin (Lee, 1991). Lipovitellin provides materials and energy for the larvae when they first hatch from the egg and before they begin to feed on external food. It has been suggested that, in at least some species of polychaetes, lipovitellins are synthesized in eleocytes, secreted into the coelomic fluid and taken up by the developing oocytes (Baert, 1986; Baert and Slomianny, 1987; Porchet, 1984; Porchet et al., 1989).

Partial characterization of several polychaete lipovitellins has been carried out (Baert, 1985, 1986; Baert et al., 1984; Fischer and Schmitz, 1981; Lee, 1991). A 420-kDa lipoglycoprotein was isolated from the oocytes of *Nereis virens* and *Perinereis cultrifera* (Dhainaut et al., 1984; Fischer and Schmitz, 1981). A similar 380–390-kDa protein was isolated from immature oocytes of *P. cultrifera*; as the oocytes matured, this large lipoglycoprotein was proteolytically cleaved into five smaller peptides (98, 83, 22, 20 and 16 kDa) (Baert, 1986, 1988).

Neanthese arenaceodentata was selected for characterization of its lipoprotein composition because of its large yolk-laden ova (400–500 μm). The abundant yolk present in each ovum is necessary to supply energy during the early development as the *N. arenaceodentata* embryos remain within the male parent's tube until they reach the 21 segment stage (Davis and Reish, 1975; Rouse, 1992). At that point, the embryos leave to commence independent

* Corresponding author. Tel.: +1 912 5982494; fax: +1 912 5982310.
E-mail address: dick@skio.peachnet.edu (R.F. Lee).

1096-4959/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2004.11.002

feeding (Reish, 1957). Other reasons for the selection of *N. arenaceodentata* for our studies were their ease of culture, rapid reproduction and wide use in marine environmental quality monitoring (Davis and Reish, 1975; Pocklington and Wells, 1992; Reish and Gerlinger, 1997).

In the present study, lipovitellin and lipid droplets from fertilized eggs of *N. arenacendentata* were isolated by density gradient ultracentrifugation. This technique allowed one-step isolation of large intact lipoproteins, i.e., lipovitellin, and provided information on the hydrated density of these lipoproteins. Analytical ultracentrifugation also provides information for the calculation of sedimentation rate, particle weight and diffusion constant of isolated lipoproteins (Mills et al., 1984). Cytologic features of the oocytes and eleocytes from *N. arenaceodentata* were detailed and described.

2. Materials and methods

Eggs were collected from a culture of *N. arenaceodentata* maintained at California State University at Long Beach and originally collected from Los Angeles harbor (CA, USA) in 1964. Approximately 3000 eggs were homogenized in 0.02 M Tris buffer (pH. 7.5) containing trypsin inhibitor (0.01%) in a hand homogenizer. The homogenate was centrifuged at 8000 $\times g$ for 10 min (Sorvall RC2B centrifuge), producing a lipid layer, a supernatant and a pellet. The slightly yellowish lipid layer was drawn off with a pipette, and the homogenate was centrifuged at 100,000 $\times g$ to pellet the microsomes. The supernatant from the 100,000 $\times g$ was used to collect lipoproteins by sequential flotation through the addition of salt solutions prepared according to the methods described in Lindgren (1975). Solution densities were verified by refractometry using an Abbe refractomer (Bausch and Lomb). The first centrifugation was 1.063 $\times g$/ml followed by adjustment of homogenate to 1.21 $\times g$ of solid potassium bromide followed by 40 h of centrifugation at 100,000 $\times g$. The floating layers of lipoprotein were removed and dialyzed for 24 h at 4 °C against 0.22 M sodium chloride containing 1 mM EDTA and 2 mM sodium azide. After dialysis, lipoproteins were run on vertical slab gels (7% polyacrylamide, 0.1% sodium dodecyl sulfate, 0.8% mercaptoethanol; SDS-PAGE) following the procedures of Laemmli (1970). Molecular weights of the peptides were determined using a series of proteins of known molecular weight (Weber and Osborn, 1969).

Lipids were extracted by the procedures described by Bligh and Dyer (1959). Different lipid classes were separated on silica-coated Chromarods and quantified on an Iatroscan Mark IV equipped with a flame ionization detector using procedures described by Parrish (1987). Reference solutions of cholesterol, triolein and phosphatidyl choline were used to identify and quantify lipid classes in lipoprotein and lipid layer preparations. Total lipid was the sum of all lipid classes eluted from the Chromarods. Some lipid samples were also run on silicic acid thin-layer plates using a solvent system of petroleum ether/ethyl ether/acetic acid (80:20:1). Protein was determined by the procedures described by Bradford (1976) and carbohydrate by the phenol–sulfuric acid method described by Dubois et al. (1956).

For microscopy studies, mature and slightly less mature female *N. arenaceodentata* were fixed in zinc formalin, processed in the standard manner for routine light microscopy and embedded in paraffin. Five-micron sections were cut, mounted on glass slides, stained, coverslipped and examined by one of us (ANW).

3. Results

3.1. Isolation and characterization of lipovitellin

The primary components of *N. arenaceodentata* egg were protein and lipid with lesser amounts of carbohydrate (Table 1). After homogenization and centrifugation of eggs, a lipid layer was noted and was collected and extracted. This lipid layer, presumably associated with lipid droplets in the eggs, was composed primarily of triacylglycerols. Isolation of lipoproteins from the egg cytosol (supernatant after 100,000 $\times g$ centrifugation of egg homogenate) showed an absence of lipid and protein in very low density and low-density classes (d<1.06 g/ml). Between 80% and 90% of the protein in the egg cytosol was associated with the high-density lipoprotein class (HDL_3 density: 1.12–1.21 g/ml). Between 35% and 37% of the egg protein was associated with this high-density lipoprotein. This lipoprotein was 57% protein, 42% lipid and 1% carbohydrate. The lipid moiety of the lipoprotein was composed of triacylglycerol (3% of lipid), cholesterol (3% of lipid) and phospholipids (92% of lipid) (Table 1). SDS-PAGE analysis of the isolated egg

Table 1

Egg component	Concentration±S.D. (μg/egg)
Composition of *Neanthes arenaceodentata* eggs	
Three groups of 1000 eggs were used for the analysis; S.D.—standard deviation (n=3)	
Protein	4.1±0.6
Carbohydrate	1.1±0.3
Lipid	3.1±0.4
Triacylglycerols	0.8±0.2
Phospholipid	2.2±0.3
Lipid	**Percent of total lipid (±SD)**
Lipid composition of lipoprotein (lipovitellin) isolated from *Neanthes arenaceodentata* eggs. Analysis of lipovitellin isolated from the three groups of 1000 eggs (n=3)	
Triacylglycerols	3±1
Cholesterol	3±2
Phospholipid	92±11