UNDEGRADED VITELLOGENIN POLYSOMES FROM FEMALE INSECT FAT BODIES

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SUMMARY. Fat body cells of vitellogenic females of the cockroach Leucophaea maderae contain a prominent population of large polysomes of approximately 35-40 ribosomes whereas fat bodies of non-vitellogenic females or males of any age do not have these polysomes. Anti-vitellogenin recognizes newly synthesized nascent vitellogenin associated with these large polysomes. Adenosine labelled RNA is likewise precipitated by anti-vitellogenin primarily in the region of this class of polysomes. It is concluded that the class of large polysomes represents the vitellogenin polysomes.

Vitellogenins are defined as the precursors to the predominant yolk proteins (1). They are synthesized extraovarially and taken up by the growing oocytes via pinocytosis. During egg growth vitellogenins are produced in the fat bodies of insects or the livers of amphibians and birds at extremely high rates. In insects about 90 percent of the yolk proteins consist of vitellin, the yolk protein which is derived from vitellogenin. It has been found that vitellogenin synthesis is directed by hormones, i.e. estrogens in birds (2) and amphibians (3) or juvenile hormone in the majority of insect species (4,5). In the cockroach Leucophaea maderae (6,7) and the locust Locusta migratoria (8) vitellogenins are synthesized on rough surfaced endoplasmic reticulum and secreted by vectorial discharge into the cisternae. This mechanism of secretion is similar to that shown for other exportable proteins (9,10). This implies that the specific polysomes synthesizing vitellogenin must be located on the endoplasmic reticulum of the fat body cells of vitellogenic females. However, to date identification of specific polysomes synthesizing insect vitellogenin has not been reported. The characterization of such specific polysomes is essential for an understanding of the process of vitellogenin synthesis. It is of particular interest because of the involvement of a hormone that directs the massive production of an identifiable protein in a fully differentiated tissue. I have now obtained

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evidence for the existance of such a specific class of vitellogenin synthesizing polysomes in the fat bodies of the female cockroach Leucophaea.

MATERIAL AND METHODS

Colonies of the cockroach Leucophaea maderae are maintained at 26°C and approximately 75% relative humidity. Newly emerged females were collected from these colonies and set aside for later use.

For polysome preparations 70-80 mg of fat bodies were homogenized in Tris-HCl buffer (200mM) at pH 9.0 containing 200mM sucrose, 400mM KCl, 35mM $MgCl_2$, 1% Triton X-100, and 25mM EGTA (ethyleneglycol-bis (β-aminoethyl ether) N,N'-tetraacetic acid) (11). Following two centrifugations at 10,000 g for 10 minutes each, the supernates were directly layered onto sucrose gradients of 15 to 60 percent. The gradients were made in Tris-HCl buffer (40mM) at pH 9.0 and contained 200mM KCl, 35mM $MgCl_2$, and 5mM EGTA. After three hours of centrifugation in a SW 27.1 rotor at 76,000 g the gradients were emptied while absorbancy was monitored at 254 nm using an ISCO UV analyzer. Two ml fractions were collected. Aliquots of each of the fractions were diluted with buffer before anti-vitellogenin or antiserum to male hemolymph were added. After one hour incubation at 37°C, carrier protein (diluted female hemolymph) was added and then refrigerated over night. The precipitates were centrifuged down, washed once in buffer and trichloroacetic acid, and then redissolved in NaOH for counting.

RESULTS

In a typical absorbancy profile of polysomes obtained from vitellogenic females a prominent peak of undegraded polysomes is seen in the lower portion of the sucrose density gradient (Fig. 1). These polysomes are characteristic for fat bodies from females during the period of most intense vitellogenin synthesis (egg size of 2 to 4 mm). They can be completely destroyed by ribonuclease (1 µg/ml), or treatment of the 10,000 g supernate with EDTA (10mM). This prominent absorbancy peak was never observed in homogenates from fat bodies of non-vitellogenic females or males of any age.

Since the appearance of the unique absorbancy peak is correlated with vitellogenesis, I suspected that it contained a class of polysomes involved in vitellogenin synthesis. To show this fat bodies from vitellogenic females were collected 24 hours after an injection of 40 µCi of ^{3}H-adenosine and 80 mg of tissue used for sucrose density gradient analysis. After fractionation, aliquots from each fraction were treated with anti-vitellogenin or antiserum to male hemolymph, and the precipitates processed as before (12). Anti-vitellogenin recognized antigens primarily associated with the class of

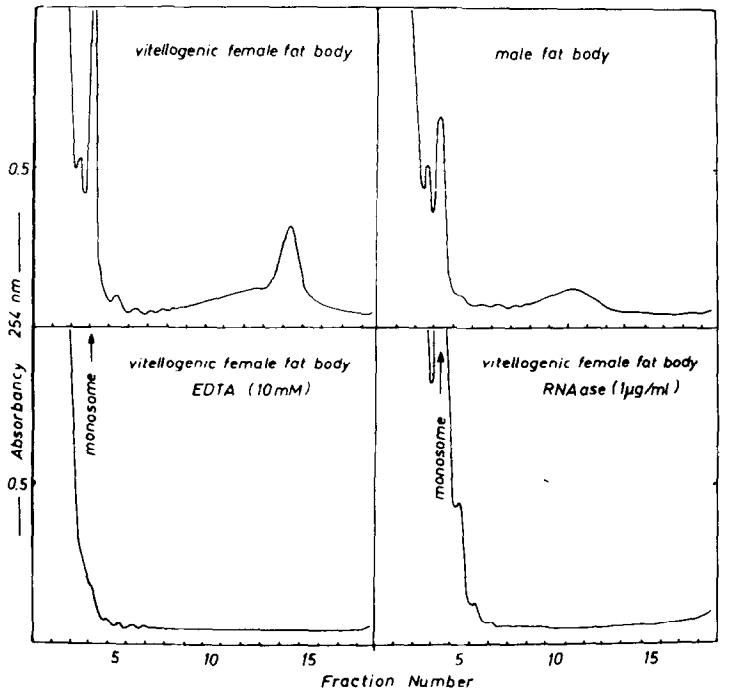
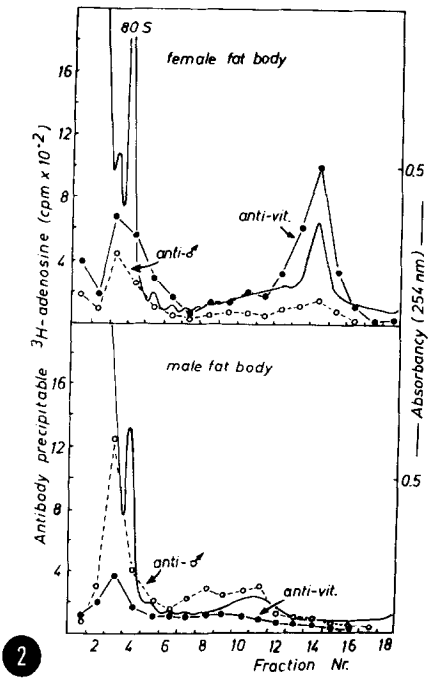


Fig. 1. Polysome profiles of fat bodies from vitellogenic females and males. In all cases 80 mg of fresh fat body tissues were used. Fat bodies were homogenized in a medium containing EGTA. After centrifugation at 10,000 g disodium EDTA or ribonuclease (bovine pancreas) was added to the supernates and then layered directly onto the sucrose gradients.

labeled large polysomes (Fig. 2). Male antisera did not precipitate more than 10-15 percent of the label precipitable with anti vitellogenin. This artifact is the result of non-specific trapping of counts due to the addition of carrier antigens to the incubation media. In fractions containing the large ribosomal subunits and the monosome a certain amount of label was also precipitated with anti-vitellogenin. This may indicate a certain degree of polysomal degradation during the preparation, the degradation products still carrying nascent polypeptides. These data provide strong evidence that the large polysomes represent the class of vitellogenin polysomes. A polysome size of 35 to 40 ribosomes was estimated by extrapolation of the log-linear relationship between ribosome monomeric units and distance of migration in the sucrose density gradient.



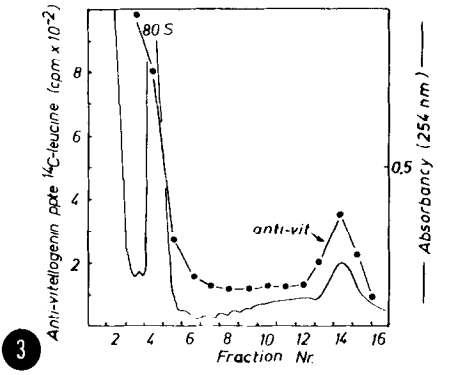


Fig. 2. Polysome profiles of fat bodies from vitellogenic females and males. The animals had been injected with 40 µCi of [2,8-^{3}H]-adenosine (spec. act. 30.4 Ci/mmol, NEN) 24 hours prior to collecting the tissues. After sucrose density gradient centrifugation and fractionation aliquots of the fractions were treated with anti-vitellogenin or antiserum to male hemolymph and the precipitates processed as before (12).

Fig. 3. Polysome profile of fat bodies from vitellogenic females which had been injected with 1 µCi of [^{14}C(U)]-leucine (spec. act. 314 mCi/mmol, NEN) two hours prior to collection of the tissues. The labelled vitellogenin of each fraction was precipitated by treatment with anti-vitellogenin. Since pelleting of the polysomes prior to sucrose density gradient centrifugation had to be avoided, large amounts of vitellogenin freed during homogenization and dissolution of the microsomal vesicles now float on top of the gradient.

Fat bodies prepared from males with the same procedure as described above did not yield the very large polysomes precipitable by anti-vitellogenin (Fig. 2). Anti-vitellogenin precipitated, however, a certain amount of label in the top of the gradient; this can be considered an artifact, since male fat bodies are not known to produce vitellogenin *in vivo* under any circumstances (12).

^{14}C-Leucine labeled polypeptides precipitable by anti-vitellogenin were found almost exclusively associated with the class of large polysomes. The

radioprofile followed very closely that of the absorbancy at 254 nm in this area (Fig. 3). Thus, both newly synthesized RNA and protein can be precipitated by anti-vitellogenin nearly entirely in the region of the large polysomes.

During the many attempts to identify the vitellogenin polysomes it became apparent that pelleting of microsomes or polysomes at 100,000 g either prior to or after detergent treatment was detrimental: the large polysomes were degraded even when EGTA was contained in the media. Freezing of the intact tissues or the 10,000 g supernate with or without EGTA also destroyed these large polysomes. The use of heparin (1 mg/ml) or diethylpyrocarbonate (0.05%) as nuclease inhibitors in homogenization and gradient media in the absence of EGTA was ineffective in producing the specific polysomes.

The unique polysome profile from fat bodies of vitellogenic females suggests that most of the translational activity of these tissues occurs on the very large polysomes. During the maximum growth phase of the oocytes about 87 percent of the proteins synthesized and released into the hemolymph is vitellogenin (Table 1). While 87 percent of the labeled proteins of the hemolymph is vitellogenin, quantitatively it makes up only about 5 percent of the total proteins. Oocytes preferentially take up the newly synthesized and released vitellogenin. Vitellogenin synthesis and release declines shortly before ovulation to a low level and ceases completely at ovulation.

DISCUSSION

The reliable identification of vitellogenin polysomes was made possible by the use of the Ca chelator EGTA (11). Omission of polysomal pelleting prior to gradient centrifugation or freezing of the tissues was, however, equally important. None of the published procedures on polysome isolation were successful. This points to the fact that the fat bodies contain large amounts of Ca dependent RNases and that vitellogenin polysomes of insects are extremely sensitive to degradation during high speed centrifugation. This sensitivity of the polysomes to degradation may argue against the notion

TABLE 1.

Incorporation rates of ^{14}C-leucine into serum proteins of vitellogenic females.

Oocyte size mm	Number of animals	Total serum proteins		Vitellogenin	
		cpm/ 1 µl	spec. act. cpm / µg protein	cpm/ 1 µl	spec. act. cpm/µg protein
2.3 - 4.0	6	359 ± 91*	9.7 ± 2.4	322 ± 94	232 ± 74[b]
5.0[a]	3	49 ± 11	2.7 ± 1.6	34 ± 19	--[c]

Animals were injected with 1 µCi ^{14}C-leucine 2 hr prior to bleeding. Proteins of aliquots of the sera were precipitated with either 10 percent trichloroacetic acid or anti-vitellogenin and the precipitates processed as previously described (12). The amount of vitellogenin in the samples was determined by rocket immunoelectrophoresis. This technique is sensitive enough to detect vitellogenin quantitatively in a sample of 0.1 µl of serum from a vitellogenic female.

* Mean ± S.E.

[a] Fully grown oocytes.

[b] This figure is based on only 3 of the samples, because the other 3 samples were accidentally lost.

[c] No determinations of the amounts of vitellogenin in these samples were made.

that the prominent peak in the lower portion of the sucrose density gradient represents an aggregation of small polysomes. Aggregation of polysomes caused by the 35 mM Mg^{++} in the media is unlikely, since on the one hand, male tissues or those of non-vitellogenic females treated identically did not yield this size class of polysomes, and on the other, lower Mg^{++} concentrations (10mM) did not substantially affect the absorbancy profiles.

Recently, large vitellogenin polysomes of 30-40 ribosomes have been identified in avian liver by immunoprecipitation (13). Also, liver of estrogenized Xenopus males contained vitellogenin polysomes of 30 ribosomes (14). In either case the size of the primary translation product of these polysomes appears to be in the order of 200,000 daltons. The primary translation product of Leucophaea vitellogenin polysomes is not known for

certain, but considering the polysome size one may predict that it is not too dissimilar to that known for Locusta (8), i.e. approximately 250,000 daltons.

The unique polysome profile of the fat bodies of reproductively active females points to the fact that this tissue is making primarily vitellogenin at this time. It is a ready source for the isolation of the vitellogenin messenger.

For any further understanding of the detailed mechanisms of vitellogenin biosynthesis in the fat bodies of an insect the availability of the vitellogenin mRNA is crucial. With the successful identification of the vitellogenin polysomes we are now in the position to perform the critical experiments.

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