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THE ROLES OF HAEMOLYMPHATIC LIPOPROTEINS IN THE OOGENESIS OF *RHODNIUS PROLIXUS*

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The fates of purified ^{32}P-vitellin and ^{32}P-lipophorin were followed in vitellogenic females of Rhodnius prolixus. *While the radioactivity from ^{32}P-vitellin 6 hours after injection was found almost exclusively in the ovary, the radioactivity from injected ^{32}P-lipophorin was found distributed among several organs. In the ovary, the radioactivity from ^{32}P-vitellin was associated with the contents of the yolk granules. ^{32}P-lipophorin delivered a great amount of radioactive phospholipids to the ovary with no accumulation of its protein moiety, as observed after its iodination with ^{131}I. The delivery of phospholipids was inhibited at 0ºC and by the metabolic inhibitors, sodium azide and sodium fluoride. Comparison of the radioactivity incorporation from ^{32}P-lipophorin with that of ^{14}C-inulin suggests that the ^{32}P-phospholipids from lipophorin are not taken up by fluid phase endocytosis. The data presented here are compatible with the concept of lipophorin as a carrier of lipids in insects and provide evidence that lipophorin transports phospholipids as shown previously for other classes of lipids. The utilization by the oocytes of the phospholipids transported by lipophorin is discussed.*

One of the most impressive aspects of oogenesis in insects is the rapid accumulation in the oocytes of the main yolk protein, a lipoglycoprotein called vitellogenin. The process of oogenesis is very complex and involves the coordination of several organs. Vitellogenin is produced by the fat body, secreted into the haemolymph and taken up into the growing oocytes by endocytosis. The process of producing an egg is orchestrated by hormones, which control the activity of the organs involved (Engelmann, 1979; King & Buning, 1985).

The specificity of the vitellogenin uptake process has been recognised (Telfer, 1954; Roth et al., 1976), but the biochemical basis of recognition remains unclear, although some information about the morphological events that accompany this process is available. Vitellogenin binds to a specific receptor at the surface of the oocyte and is internalised into coated vesicles (Roth & Porter, 1964; Anderson, 1971; Roth et al., 1976; Telfer et al., 1982; Bradley, 1983; Oliveira et al., 1986; Osir & Law, 1986). The association of coated vesicles with specific endocytosis was first observed in the oocytes of *Aedes aegypti* by Roth & Porter (1964). Soon after the endocytic vesicles are formed they lose their coat and a great number of small vesicles fuse, giving rise to yolk granules as described by Roth et al. (1976). By this process the yolk protein, which is composed of lipid (5-15%), carbohydrate (1-14%) and protein (70-90%) (Chino et al., 1977; Chen et al., 1978) is internalised and stored until use by the future embryo. The name vitellin (VT) is used to refer to the yolk protein accumulated in the oocyte to differentiate it from vitellogenin present in the haemolymph.

A great quantity of lipid is also found in the egg (Wiemerslage, 1976; Lubzens et al., 1981). Chino et al. (1977) proposed that the lipids accumulated in the oocytes are transported from other tissues to the ovary by lipophorin, the major haemolymphatic lipoprotein, known to carry several classes of lipids between tissues (Chino et al., 1969; Chino & Gilbert, 1971; Chino & Kitazawa, 1981; Katase & Chino, 1982; Chino, 1985).

Telfer et al. (1982) calculated for *Hyalophora cecropia* that every 3 hours the surface of an oocyte has to generate 300 times its initial area to satisfy the requirement for membranes necessary for vitellogenin uptake.

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TABLE I

Comparison of radioactivity incorporation by different organs of vitellogenic females of *Rhodnius prolixus* following injection of ^{32}P-vitellin and ^{32}P-lipophorin

Lipoprotein	Fat body	Muscle	Digestive system	Ovary
^{32}P-vitellin	0.27%	1.27%	0.65%	17.90%
^{32}P-lipophorin	3.28%	1.61%	5.54%	9.48%

Purified ^{32}P-vitelin (50,000 cpm in 2 μl of 0.15 M NaCl) and ^{32}P-lipophorin (42,300 cpm in 5 μl of 0.15 M NaCl) were injected into vitellogenic females. After 7 hours at 28°C the fat body, thoracic, muscles, digestive system and ovary of each animal were dissected, extensively washed in 0.15 M NaCl, and homogenised. The radioactivity incorporated by each organ was determined by scintillation counting. The values express the percentage of the total radioactivity injected and represent the means of 4-6 determinations. Purified ^{32}P-vitellin and ^{32}P-lipophorin were obtained as described elsewhere (Oliveira et al., 1986; Gondim, 1986).

Although a membrane recycling mechanism must be operating during fusion of endosomes, a great amount of new membrane must be made by the oocyte to take care of the yolk granule formation. In a previous work Masuda & Oliveira (1985) described a method for labelling vitellin and lipophorin with ^{32}P by feeding the insects with blood enriched with ^{32}Pi. As a first approach to understanding the mechanism utilised by the ovary to satisfy the requirement for new membranes, we followed the fate of radioactivity from ^{32}P-vitellin and ^{32}P-lipophorin, lipoproteins known to carry radioactive phospholipids, in vitellogenic females.

RESULTS AND DISCUSSION

After purification of metabolically labelled ^{32}P-vitellin (^{32}P-VT) and ^{32}P-lipophorin (^{32}P-LP) on a Kbr ultracentrifugation gradient as described by Oliveira et al. (1986), the fates of these two lipoproteins were analysed following injection into vitellogenic females of *Rhodnius prolixus*. In order to allow a comparison of incorporation of radioactivity from ^{32}P-vitellin and ^{32}P-lipophorin into different organs, the data are expressed as percentages of the total radioactivity injected. The results in Table I clearly show that while the ^{32}P-VT was taken up almost exclusively by the ovary, the radioactivity from ^{32}P-LP was more evenly distributed among different organs. It is worth noting that while radioactivity in ^{32}P-vitellin is found in the protein, carbohydrate and lipid (phospholipids) moieties (Masuda & Oliveira, 1985), the radioactivity in ^{32}P-lipophorin is associated exclusively with the lipid moiety (Gondim, 1986). Thus the transfer of radioactivity from ^{32}P-lipophorin means transfer of phospholipids.

As vitellogenin undergoes no or only minor modifications after being taken up by the oocytes (Kunkel & Nordin, 1985), vitellin can be used in the place of vitellogenin in experiments for uptake measurements (Oliveira et al., 1986). Analysis of the fate of ^{32}P-vitellin in the oocyte 7 hours after injection into a vitellogenic female showed that it was found in the granules as expected, associated primarily with the granule contents rather than with their membranes (Table II).

TABLE II

Analysis of the radioactivity distribution in yolk granules of *Rhodnius prolixus* following injection of ^{32}P-vitellin

Fraction of yolk granules	C.P.M.	(%)
Precipitate (granule membranes)	2,030	(11.4%)
Supernatant (granule contents)	15,811	(88.6%)

Seven hours after injection of purified ^{32}P-vitellin into vitellogenic females, their ovaries were dissected, extensively washed in 0.15 M NaCl and the yolk granules released by gently squeezing the ovaries with a loose pestle in a homogeniser. The released material containing the granules was centrifuged at 400 x g for 1 minute. The precipitate was enriched in yolk granules, as monitored by optical microscopy and by polyacrylamide gel electrophoresis. The yolk granules were disrupted by freezing and thawing followed by a strong agitation on a Vortex mixer for 2 minutes. This suspension was centrifuged in a Beckman Airfuge at 100,000 x g for 1 hour. The precipitate was considered to contain membranes of yolk granules and the supernatant their contents. The radioactivity present in each of these fractions was estimated by scintillation counting.

TABLE III

Comparison of radioactivity incorporation by ovaries of *Rhodnius prolixus* following injection of ^{32}P-vitellin, of radioactive lipophorin labelled with ^{32}P or ^{131}I, and of ^{14}C-inulin

Injections	28°C	0°C
A) ^{32}P-VT	19.0%	1.0%
B) ^{32}P-LP	7.2%	1.5%
C) ^{32}P-LP + NaF	1.5%	–
D) ^{32}P-LP + NaN_3	0.8%	–
E) ^{131}I-LP	2.3%	2.5%
F) ^{32}P-I-LP	7.4%	–
G) ^{14}C-inulin	2.2%	–

Vitellogenic females were injected with: (A) 16,340 cpm of ^{32}P-vitellin in 3 μl (B) 15,000 cpm of ^{32}P-lipophorin in 5 μl. (C) 2 μl of 0.5 M sodium fluoride followed by 58,000 cpm of ^{32}P-lipophorin in 4 μl. (D) 2 μl of 0.5 M sodium azide followed by 58,000 cpm of ^{32}P-lipophorin in 4 μl. (E) 15,000 cpm of ^{131}I-lipophorin in 2 μl. (F) 7,000 cpm of ^{32}P-lipophorin iodinated with non-radioactive iodine (^{32}P-I-LP) in 6 μl. (G) 70,000 cpm of ^{14}C-inulin in 2 μl. All substances injected were in 0.15 M NaCl. After the injections, the females were maintained at 0°C or 28°C and 6 hours later the ovary of each animal was dissected, extensively washed in saline and homogenised, and the radioactivity incorporated determined by scintillation counting for ^{32}P-labelled molecules or by gamma counting for ^{131}I-labelled molecules. Purified ^{32}P-vitellin and ^{32}P-lipophorin were prepared as described elsewhere (Oliveira et al., 1986; Gondim, 1986). Iodination procedure was as described by Mc Conahey & Dixon (1980). The values are expressed as percentage of total radioactivity injected and represent the mean of 4-6 determinations.

A comparison of the uptake of radioactivity from ^{32}P-VT and from ^{32}P-LP by the ovary shows that both are taken up at 28°C and in both cases this is impaired at 0°C (Table III), suggesting dependence on active metabolism. This is reinforced by the inhibition of the transfer of radioactivity from lipophorin to the ovary at 28°C in the presence of fluoride and azide. Because the transfer of radioactivity from lipophorin to the ovary occurred during vitellogenesis, that is simultaneously with the uptake of vitellogenin, we could not discard the possibility of lipophorin being taken up in the fluid phase of vitellogenin endocytic vesicles. In order to check this possibility the uptake of lipophorin was compared with that of ^{14}C-inulin, a substance commonly used to measure the fluid phase endocytosis. The accumulation of radioactivity when ^{32}P-lipophorin was injected was several times greater than when ^{14}C-inulin was injected (Table III), suggesting different pathways for endocytosis of these two substances. This conclusion is reinforced by the demonstration by Hayakawa (1987) of a specific receptor for lipophorin in the flight muscle of *Locusta migratoria* that mediates the transfer of diacylglycerols. Although the existence of a specific receptor for lipophorin in the ovary still has to be demonstrated, it is reasonable to assume its existence in all tissues that export or import lipids by interacting with lipophorin.

In the preceding experiments the lipophorin was labelled with ^{32}P exclusively in the phospholipid moiety, as shown by Gondim (1986). Therefore only the uptake of phospholipids was measured. To study the fate of the protein moiety of lipophorin, this lipoprotein was labelled with radioactive iodine by the method of chloramine T (McConahey & Dixon, 1980). The result in Table III showed a very low percentage of radioactivity incorporation, similar to that observed at 0°C. In order to check whether the iodination procedure had interfered with its physiological function, the ^{32}P-lipophorin was iodinated with non-radioactive iodine by the same method and the transfer of radioactivity measured (Table III). In this case the incorporation of radioactivity was comparable to the control (non-iodinated ^{32}P-lipophorin), indicating that the iodination procedure had not interfered in the process of phospholipid transfer to the ovary. These results show that phospholipids are delivered to and accumulated in the oocyte but that the protein moiety is not accumulated, in accordance with the concept of lipophorin as a reusable shuttle for lipids. Masuda & Oliveira (1985) observed in polyacrylamide gels that egg extract does not show accumulation of the lipophorin polypeptides.

Taken together these data are compatible with the general description that lipophorin, the lipoprotein that delivers lipids to the oocytes, now including phospholipids, together with vitellin are used to build the egg and that during development, the vitellin and the lipids accumulated during oogenesis are used by the embryo.

If we look at the oocyte as a cell specialised for storage, one that needs a lot of phospholipids to accomodate an impressive amount of yolk protein inside the granules, it is reasonable to assume that the phospholipids delivered to the oocytes by lipophorin during vitellogenesis are used to make membranes. Although we have not measured the synthesis of phospho-

lipids by the oocytes, it can be calculated that the amount of phospholipids delivered by lipophorin is high enough to accommodate substantial membrane synthesis (Gondim, 1986).

The question of whether the protein moiety is internalised by the oocyte and secreted back to the haemolymph after unloading lipids in the oocytes or whether the transfer of phospholipids occurs at the surface of the cell without internalization remains to be answered. Clearly, however, phospholipids are delivered to the oocytes without accumulation of the protein moiety of lipophorin.

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