INTER-ORGAN HEMOLYMPHATIC TRANSPORT OF FREE FATTY ACIDS, TRIACYLGLYCEROLS AND PHOSPHOLIPIDS IN THE FRESHWATER BIVALVE, *DIPLODON DELODONTUS*

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Abstract—1. The inter-organ transport of free fatty acids, triacylglycerols and phospholipids in the hemolymph, as well as their distribution in tissues of the bivalve mollusc *Diplodon delodontus*, were studied.

2. Plasma, hemocytes and tissues were obtained 4½ hr after injecting labelled palmitic acid into the posterior adductor muscle.

3. Labelled palmitic acid in the free form or esterified into triacylglycerols and phospholipids, was detected in both plasma and hemocytes.

4. The free fatty acids and phospholipids are mainly transported by three plasma lipoproteins with low (LDL), high (HDL) and very high (VHDL) density characteristics, respectively.

5. Triacylglycerols circulate in hemolymph preferably associated with hematic cells and, to a lesser extent, they are also transported by plasma lipoproteins.

6. Highest specific radioactivities were observed in gills and digestive diverticula. They mainly correspond to the accumulation of free fatty acids and triacylglycerols.

INTRODUCTION

The fatty acid and triacylglycerol incorporation from the digestive tract to the hemolymph of the freshwater mollusc *Diplodon delondontus* was reported in a previous work (Huca *et al.*, 1984). The role of plasma and hemocytes in the transport of ingested lipids, as well as the occurrence of plasmatic lipoproteins have also been described by us (Pollero *et al.*, 1985). Recently, the diet ingested and inter-organ cholesterol transport were studied in the same species. The hemolymphatic free cholesterol is carried by means of at least two plasmatic lipoproteins with HDL and VHDL characteristics and, to a minor extent, also associated to hematic cells (Pollero, 1987).

The purpose of the present paper is to describe the mode of inter-organ circulation of free fatty acids and esterified lipids, as well as their distribution among tissues of *D. delodontus*.

MATERIALS AND METHODS

Samples

Adult specimens of *D. delodontus*, 8–12 cm long, were collected from a fresh-water pond. The animals were maintained in an aquarium with aerated water at room temperature for 5–7 days before the experiments were performed.

Labelled palmitic acid [1-^{14}C] (58 mCi/mmol) purchased from New England Nuclear was used as radioactive tracer.

Incubations with labelled palmitic acid

Two groups of molluscs (8 animals) were incubated with the radioactive tracer. Each animal was injected with 4 μCi (0.5 μmol) of 1-^{14}C ammonium palmitate into the posterior adductor muscle and maintained in the aquarium for 4.5 hr at room temperature.

After incubation, hemolymph was obtained by cardiac puncture and mantle, adductor muscles, gonad and gills were dissected. The hemolymph was centrifuged at 2000 *g* for 10 min and the hemocyte-containing pellet was washed twice with non-labelled plasma. Sonicated hemocytes and homogenized tissues were processed in order to obtain lipids.

Isolation of plasma lipoproteins

Due to the low concentration of plasmatic lipids and proteins, labelled plasma samples were concentrated using Centriflo CF-25 membranes. Lipoproteins were isolated by gradient ultracentrifugation at different densities, in a Prep-spin 75 ultracentrifuge with a swing-out rotor. Concentrated plasma samples were layered over NaBr solutions (densities 1.27 and 1.12 g/ml into the centrifugation tubes and centrifuged at 45,000 rpm (178,000 *g*) at 10°C for 22 hr. Fifteen and 18 fractions were separated from each density gradient, respectively. Density and radioactivity were determined in each fraction following the procedures previously described (Pollero, 1987). Total proteins were measured by the procedure of Lowry *et al.* (1951).

Fractions containing the radioactive peaks were isolated from the gradient centrifugations and analysed to determine radioactive lipids, lipid composition and total protein content.

Lipid extraction and analysis

Lipids from tissues, hemocytes and isolated plasma lipoproteins were extracted by the technique of Folch *et al.* (1957). Total lipids were analysed by thin layer chroma-

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Table 1. Radioactivity distribution among tissues of *D. delodontus* after injection of labelled palmitate into adductor muscle

Labelled lipid class	Adductor muscle	Gills	Digestive diverticula	Mantle	Gonad
Radioactivity (%)					
Free fatty acids	83.4 ± 6.2	42.1 ± 1.3	38.9 ± 1.0	53.4 ± 5.5	61.8 ± 6.1
Triacylglycerols	10.0 ± 4.5	35.3 ± 1.2	38.1 ± 0.4	20.7 ± 2.9	33.3 ± 2.8
Phospholipids	7.6 ± 2.7	22.8 ± 0.1	23.0 ± 0.6	25.9 ± 2.6	4.9 ± 3.3
Specific activity (dpm × 10^3/mg total lipids)	3.9 ± 1.8	9.2 ± 3.0	6.2 ± 0.5	3.8 ± 1.6	2.4 ± 1.9

Two groups of four animals each were incubated for 4½ hr with the tracer. Total lipids from each tissue were analysed by TLC and quantified by densitometry. Radioactivity in lipid classes was measured with a scanner counter. Results represent the average of two determinations ± extreme deviation of the mean.

tography (TLC) and quantified by densitometry (Blank *et al.*, 1964). The distribution of radioactivity among separated lipids was determined in the TLC plates with a scanner counter apparatus (Berthold LB 2723). The procedures for analysis and radioassay of lipids have been described in previous papers (Pollero *et al.*, 1983, 1985; Pollero, 1987).

RESULTS AND DISCUSSION

Distribution of labelled lipids among tissues

Four and a half hr after labelled palmitate injection into the adductor muscle, radioactivity was found in other tissues and hemolymph. Table 1 summarizes the distribution of radioactivity in free fatty acids, triacylglycerols and phospholipids.

The free fatty acids in each tissue had a higher radioactivity than any of the esterified acyl lipid classes, suggesting a direct hemolymphatic transport as free acids. A similar behaviour for free fatty acids absorbed from the digestive tract was reported in oysters after feeding experiments (Allen and Conley, 1982).

The lipids in the gills and digestive diverticula attained a higher specific activity than those of the other tissues. This finding may signify an active lipid delivery via hemolymph from the place of injection (adductor muscle) toward these organs. Moreover, gills and digestive diverticula also seem to be two metabolically active organs, since significant quantities of labelled triacylglycerols and phospholipids were found. In contrast, gonad tissue showed high radioactivity only in triacylglycerols (33%), suggesting a lipid storage function for this tissue to supply the energy-rich material for gamete maturation. This conclusion is consistent with the increase in the triacylglycerol content of the gonad tissue observed in *D. delodontus* during the gametogenesis (Pollero *et al.*, 1983). The metabolic activity of the adductor muscle concerning lipid biosynthesis was comparatively low, since most of the radioactivity (83%) remained in free fatty acids.

Despite the former considerations about the biosynthetic capacity of different tissues to incorporate fatty acids into acyl lipids, an interorgan circulation of free fatty acids, triacylglycerols and phospholipids was also found.

Free fatty acid, phospholipid and triacylglycerol transport in hemolymph

The radioactivity recovered in hemolymph appeared both in plasma and hemocytes. The total lipids from each hemolymphatic component were separately analysed by TLC and the distribution of the radioactivity on the plates was determined. The occurrence of labelled free fatty acids, triacylglycerols and phospholipids, in plasma as well as in hemocytes was found.

When plasma samples were ultracentrifuged on NaBr δ1.27 g/ml solution, the total protein and radioactivity measurements gave the results shown in Fig. 1. The maximum in the protein profile (fractions

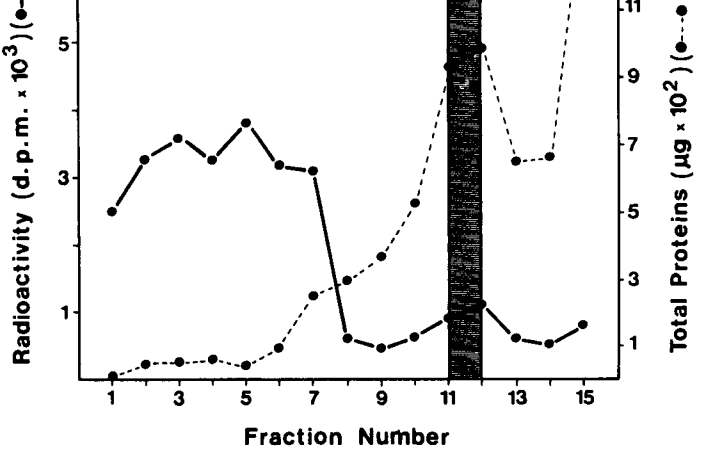

Fig. 1. Radioactivity and protein distribution in plasma fractions obtained by gradient density ultracentrifugation in NaBr 1.27 g/ml. Labelled plasma was obtained after 1-^{14}C palmitate injection into *D. delodontus* posterior adductor muscle. Shadowed bar corresponds to fractions containing VHDL which were analysed.

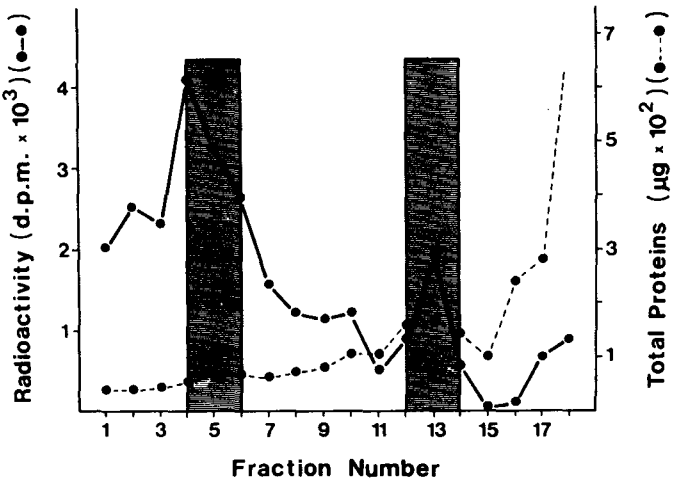

Fig. 2. Radioactivity and protein distribution in plasma fractions obtained by gradient density ultracentrifugation in NaBr 1.12 g/ml solution. Labelled plasma was obtained after 1-^{14}C palmitate injection into *D. delodontus* posterior adductor muscle. Shadowed bars correspond to fractions containing LDL and HDL, respectively, which were analysed.

11–12) is coincident with a peak in the labelling profile, and it corresponds to a lipoprotein fraction with a hydrated density between 1.250 and 1.258 g/ml, which may be considered as a very high density lipoprotein (VHDL).

To get a better resolution of the minor density zone of the gradient which presented a significant amount of radioactivity, plasma samples were ultracentrifuged over a NaBr δ 1.12 g/ml solution and divided into 18 fractions. The protein and radioactivity profiles obtained showed three coincident maxima (Fig. 2). The maximum observed at the major density zone (fraction 18) possibly corresponds to the above-mentioned VHDL. The peak in the intermediate zone of the gradient (fractions 12–14) corresponds to a lipoprotein fraction with a hydrated density between 1.090 and 1.115 g/ml, which may be considered as a high density lipoprotein (HDL). The major peak in the radioactivity profile can be observed in a zone of relatively minor density (fractions 4–6). It shows a hydrated density of 1.040–1.054 g/ml and corresponds to a lipoprotein fraction that may fall into the category of a low density lipoprotein (LDL).

It has been previously demonstrated that in *D. delodontus* there appear two plasmatic lipoproteins with HDL and VHDL characteristics, which incorporate and transport most of the cholesterol in the interorgan circulation as well as the cholesterol absorbed from the digestive tract (Pollero, 1987). The present study revealed that the same lipoproteins accomplish the interorgan transport functions of lipids other than cholesterol. But, in addition to them, a lipoprotein fraction with characteristics of LDL also participates in the plasmatic lipid transport among the organs.

The main difference between the former (Pollero, 1987) and the present study lies in the density used for the gradient ultracentrifugations. By using a more resolutive condition for the minor density zone, it was possible to reveal in the present case, the existence of a new lipoprotein fraction. The occurrence of a LDL in *D. delodontus* is consistent with the results of our early study about lipid transport in bivalves (Pollero *et al.*, 1985), where we found that free fatty acids absorbed from the digestive tract were mainly transported in association with a low density plasmatic fraction.

The gradient ultracentrifugation zones containing LDL, HDL and VHDL, respectively (shadowed bars in Figs 1 and 2), were separately analysed by TLC to determine their labelled lipid classes. The scanning of the radioactivity on the TLC plates showed labelled free fatty acids, phospholipids and triacylglycerols in all lipoprotein fractions, although in different proportions. Table 2 summarized these results as well as those obtained from hemocyte analysis.

About two-thirds of the hemolymph labelling appeared in plasma whereas the remaining label was found associated with hemocytes. The relative distribution of the radioactivity incorporated into the

Table 2. Labelling distribution in hemolymph lipids of *D. delodontus* $4\frac{1}{2}$ hr after injection of 1-^{14}C palmitate into adductor muscle (dpm%)

Labelled lipid class	Hemocytes	Plasma		
		LDL	HDL	VHDL
Free fatty acids	7.8	55.8	19.0	17.4
Phospholipids	18.3	65.7	13.8	2.2
Triacylglycerols	79.7	13.2	4.3	2.8
Labelling in total lipids*	30.5 ± 3.5	50.1 ± 2.5	12.9 ± 0.7	6.5 ± 0.3

*Results represent the average of two determinations ± extreme deviation of the mean.

Table 3. Lipid composition of the LDL isolated from *D. delodontus* plasma

Lipid class	Percent (w/w)
Phospholipids	44.0
Free sterols	24.5
Free fatty acids	5.2
Triacylglycerols	21.1
Esterified sterols	5.2
Total lipids	50.3
Total proteins	49.7

Lipids were separated by TLC and quantified by densitometry.

Total proteins were measured by colorimetry (Lowry *et al.*, 1951). VHDL and HDL compositions have been previously reported (Pollero, 1987).

different lipid classes, indicates that plasma lipoproteins are the principal carriers of the free fatty acids and phospholipids, whereas the plasmatic transport of triacylglycerols is comparatively of minor significance. Most of the plasma radioactivity was found incorporated into the LDL. Therefore, about 56% of the labelled free fatty acids and 65% of the labelled phospholipids are transported by this lipoprotein fraction.

Lipid composition of the LDL isolated from the *D. delodontus* plasma is shown in Table 3. Phospholipids and free sterols are the largest lipid classes. Triacylglycerols also represent an important lipid fraction, whereas free fatty acids and esterified sterols are comparatively of minor significance. The relevant role of the LDL in the plasmatic lipid transport is consistent with the proportion of its lipid moiety (50% of the lipoprotein weight), which is large in comparison with the 22.6 and 5.6% of the HDL and VHDL lipid moieties, respectively, as reported previously (Pollero, 1987). Although *D. delodontus* was isolated at a density similar to that of those LDL from other organisms, it markedly differs from them in the total lipid content. This low density lipoprotein fraction is also clearly different in structure from those of fish, insect and mammal LDL, since phospholipids and free sterols are the major lipids, whereas triacylglycerols predominate in fish and insects, respectively; esterified sterols are the major lipids in mammals LDL (Mills *et al.*, 1977; Chino *et al.*, 1969; Chapman, 1980).

Esterified sterols and triacylglycerols are known components of the hydrophobic core of most animal LDL, and alkyldiglycerids as well as hydrocarbons accomplish a similar role in LDL of certain primitive marine vertebrates (Mills and Taylaur, 1978). These non-polar lipids normally account for 47–72% of LDL total lipids (Chapman, 1980; Fremont *et al.*, 1981). In contrast, the summation of non-polar lipids in *D. delodontus* LDL reached only 25% of the total lipid moiety, the amount of esterified sterols being noticeably lower than those LDL from other origins. In view of such characteristics, this lipoprotein fraction from *D. delodontus* does not seem to be an ordinary low density lipoprotein.

Although free fatty acids and phospholipids are usually transported by plasma lipoproteins, most of the triacylglycerols and a significant amount of phospholipids circulate in the hemolymph associated with hematic cells (Table 2). This fact is consistent with our former findings in the same species, when we studied the absorption of triacylglycerols from the digestive tract (Huca *et al.*, 1984) and the *in vitro* incorporation of triacylglycerols to the hemocytes (Pollero *et al.*, 1985). Besides, it was previously shown that hemocytes can also incorporate and transport both dietary and inter-organ cholesterol (Pollero, 1987). Lipids could either be incorporated into the hematic cell or fixed to the surface, and considering the present knowledge of bivalve lipid transport, we cannot discriminate which of the two mechanisms is taking place.

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