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BIOCHEMICAL AND MORPHOLOGICAL CHARACTERIZATION OF LIPOPHORIN OF *AESHNA CYANEA* LARVAE (ANISOPTERA : AESHNIDAE)

R. BAUERFEIND and H. KOMNICK

Institut für Cytologie
Rheinische Friedrich-Wilhelms-Universität Bonn,
Ulrich-Haberland-Str. 61 a,
5300 Bonn 1, Bundesrepublik Deutschland

Lipophorin, the lipid carrier of the hemolymph, was isolated from male and female penultimate and final instar larvae by ion exchange chromatography, gel filtration or potassium bromide density gradient centrifugation. The native lipophorin is a lipoprotein of 700 kD molecular weight consisting of two glycosylated apoproteins (210 and 72 kD) and approximately 50% lipid. The major carried lipids are diglycerides, phospholipids and cholesterol, while triglycerides are minor constituents with traces of monoglycerides and free fatty acids. The lipid-loaded lipophorin is visualized as 12-15 nm particles by negative staining electron microscopy.

INTRODUCTION

In addition to their role as structural components of cellular membranes and cuticle, lipids provide an important source of metabolic fuel for insects. Due to their low water solubility, they require a transport mechanism for their distribution with the circulating hemolymph. Hemolymph lipoproteins responsible for this function have been described in several insect orders (RYAN *et al.*, 1984) and generally termed "lipophorins" because of similar biochemical properties (CHINO, 1985). The protein moiety of lipophorin is synthetized in the fat body cells and released into hemolymph (GELLISSEN & WHYATT, 1981). It is believed to serve as a reusable shuttle, transporting lipids between the sites of absorption, storage, and utilization. Studying the intestinal absorption of lipids by larval *Aeshna cyanea* (BAUERFEIND & KOMNICK, 1989) we became also interested in hemolymph lipoproteins and their role in lipid transport.

MATERIALS AND METHODS

Hemolymph of last instar larvae was collected by flushing out (CHINO, 1985) with icecold PBS (pH 7.0) supplemented with 5 mM EDTA, 50 mM reduced gluthatione, 2 mM DTT and 1 mM PMFS as a protease inhibitor. Hemocytes were sedimented (5 min, 3.800 g), and clotted material was removed by centrifugation (30 min, 120.000 g). Lipophorin was isolated from the serum by three different methods :

One step density gradient centrifugation (SHAPIRO *et al.*, 1984). Serum samples were mixed with KBr to a final concentration of 44.3% and overlayered with one volume 0.9% NaCl. After centrifugation (4 h, 10°C, 50.000 rpm, Beckman Ti 50.2 fixed angle rotor) lipophorin fractions were removed and dialyzed against flushing out buffer.

Gelfiltration (VAN DER HORST *et al.*, 1987). Serum was applied to a Sephacryl S 300 sf column (Pharmacia) equilibrated and eluted with flushing out buffer at 4°C.

Low ionic precipitation followed by ion exchange chromatography (CHINO, 1985). After short dialysis against distilled water and removal of slight turbidity, serum was added all at once to 8 volumes of icecold distilled water. The resulting precipitate was sedimented at 17.000 g (30 min) and redissolved in 200 mM phosphate buffer, pH 6.0. Ion exchange chromatography was carried out with DEAE cellulose (Whatman DE 52) equilibrated with 50 mM phosphate buffer, pH 6.0. Lipophorin was eluted in the same buffer and dialyzed against flushing out medium.

RESULTS AND DISCUSSION

We have purified a hemolymph lipoprotein of larval *Aeshna cyanea*. Its physical and chemical properties (summarized in Table I) allow us to classify this protein as lipophorin (BEENAKKERS *et al.*, 1988). All three methods used yielded a homogeneous lipophorin preparation of uniform density and subunit structure. However, density gradient contrifugation was the most rapid and efficient method in terms of purity and yield, and was preferably used in the present work.

When serum samples were stained for lipids with Sudan Black B before ultracentrifugation (Fig. 1a) or isoelectric focussing (Fig. 2c, lane 2) two different lipoproteins were visualized. They correspond to the yellow and green bands present after density gradient centrifugation (Fig. 1a). The yellow band with an average density of 1.10 g/ml (Fig. 1c) was lipophorin, while the green band represented a chromoprotein (HAUNERLAND & BOWERS, 1986) with a lipidic pigment.

SDS-Page of isolated lipophorin under reducing and non reducing conditions revealed two subunits with molecular weights of 210 kD and 72 kD (Fig. 2c, lane 5), corresponding to apoLp-I and apoLp-II (RYAN *et al.*, 1984). A third light subunit (apoLp-III) was not detected. So far its presence was reported only in a few species. It was attributed a regulatory role during lipid loading, particularly in those insects which use lipid as a fuel for flight (VAN DER HORST *et al.*, 1988) [1].

Both subunits faintly stained with the PAS reaction indicating the presence of a carbohydrate moiety. Mannose was identified as one sugar component

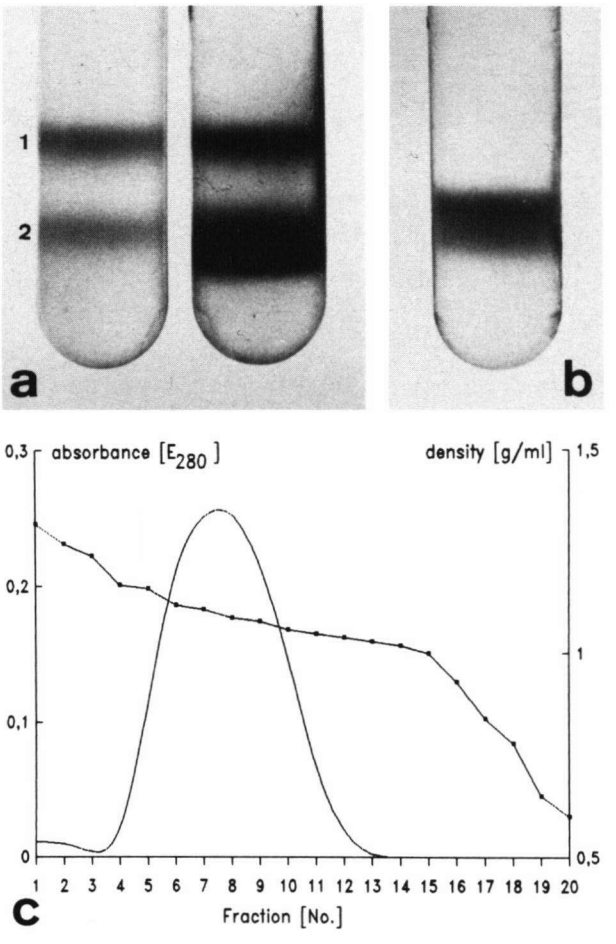

Fig. 1. Isolation of lipophorin by density gradient centrifugation ; — (a) hemolymph serum after centrifugation : unstained (left ; 1 : yellow band ; 2 : green band) and prestained with Sudan Black B (right) ; — (b) isolated lipophorin after centrifugation ; — (c) collecting of protein fractions (–●–● protein content (E_{280}) ; —— density determinated by refractometry).

[1] In addition to apoLp-I and apoLp-II which are the only apolipoproteins of *Aeshna* larvae, we found a third apolipoprotein associated with 15-18% of lipophorin of adult *A. cyanea*, which were sacrified within 3 days after emergence. ApoLp-III is watersoluble and has an apparent molecular weight of 20 kD.

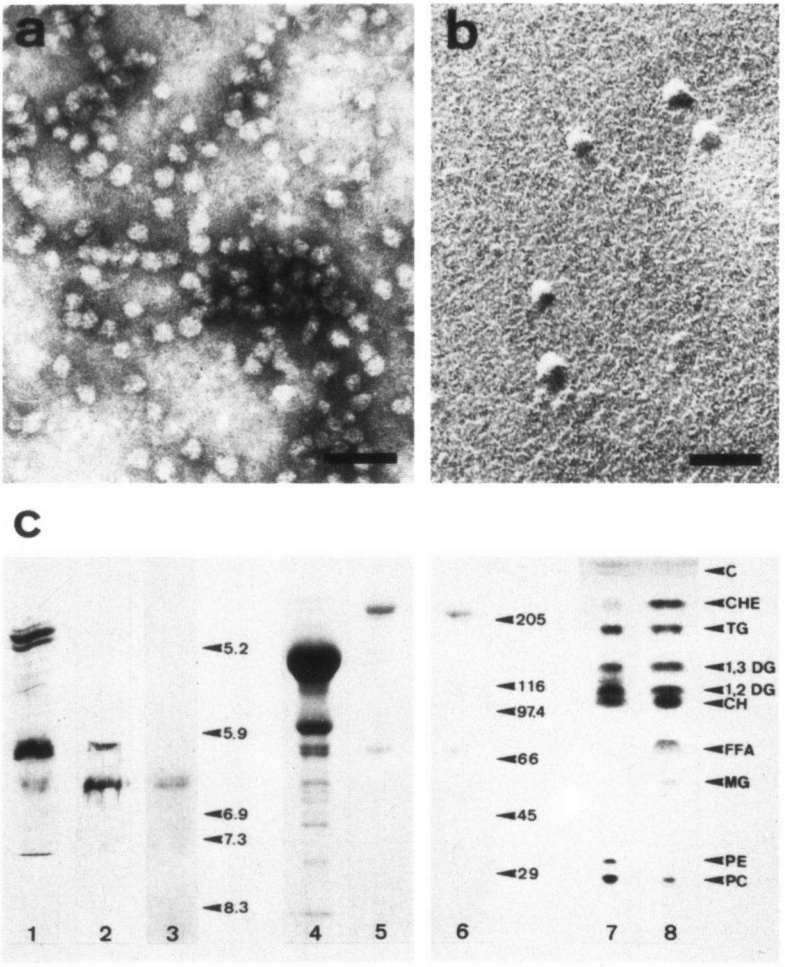

Fig. 2. Electron micrographs of isolated *Aeshna* lipophorin ; — (a) negatively stained with uranyl acetate ; the measurement of molecular size was made on 150 molecules within a certain area of the electron micrograph ; — (b) shadowed with Pt/C ; 20°. Bars 50 nm ; — (c) gel electrophoresis and thin layer chromatography (Lane 1 : isoelectric focussing of hemolymph serum stained with Coomassie Blue ; lane 2 : same, prestained with Sudan Black B ; lane 3 : isoelectric focussing of isolated lipophorin stained with Coomassie Blue ; numbers represent pI-standards. — Lane 4 : SDS-PAGE of hemolymph serum and lane 5 : isolated lipophorin showing the two subunits ; lane 6 : peroxidase-Con A staining of the two subunits ; numbers represent molecular weight standards (kD). — Lane 7 : thin layer chromatography of lipid classes of isolated lipophorin ; lane 8 : lipid standards and identified spots : C carotinoid ; CHE esterified cholesterol ; TG triacylglyceride ; 1,3 DG and 1,2 DG diacylglyceride ; CH cholesterol ; FFA free fatty acids ; MG monoacylglyceride ; PE phosphatidylethanolamine ; PC phosphatidylcholine).

by the binding reaction with peroxidase-labelled Con A (Fig. 2c, lane 6). The molecular weight of native lipophorin is between 650 and 720 kD depending on the method of determination (Tab. I). This variance seems to depend on the extent of the lipid loading (CHINO, 1985). Correspondingly, the particle size of the lipophorin molecule (Fig. 2a and b) varies between 12 and 15 nm.

The total lipid of *Aeshna* lipophorin amounts to 42-50%. Diacylglycerol, phosphatidylcholine and cholesterol are the major lipid classes (Fig. 2c, lane 7) (Tab. I). In contrast to lipophorin of other species (BEENAKKERS *et al.*, 1985) a substantial amount of triacylglycerol was also found. The yellow color of lipophorin is due to an apolar hydrocarbon, which was identified as a carotinoid by thin layer chromatography (Fig. 2c, lane 7) and absorbance spectrometry (data not shown).

Table I

Biochemical and morphological properties of isolated lipophorin of larval *Aeshna cyanea*

Native molecular weight	
by nondenaturating PAGE	680 kD
by gelfiltration	750 kD
Molecular weight of the apoproteins	
apoLp-I	210 kD
apoLp-II	72 kD
Particle size (diameter)	13.7 nm (± 1.5)
Isoelectric point	pI 6.3
Density (by refractometry)	1.10 g/ml
Lipid content	42-50%
Phospholipids	32.7%
Free fatty acid	traces
Monoacylglyceride	traces
Diacylglyceride	26.9%
Triacylglyceride	8.1%
Free cholesterol	15.5%
Esterified cholesterol	2.0%
Carotinoid	2.7%
	87.9%

The biochemical properties of lipophorin of *Aeshna* larvae largely correspond with the lipophorins of other insects studied. Our work is the first demonstration of lipophorin in *Odonata*, a phylogenetically ancient insect order.

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