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LIPID TRANSFER PROTEIN FROM *MANDUCA SEXTA* HEMOLYMPH

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SUMMARY: A hemolymph lipid transfer protein (LTP) was isolated from the tobacco hornworm, *Manduca sexta*. LTP catalyzes net lipid transfer between isolated hemolymph lipoproteins *in vitro*. An isolation procedure employing density gradient ultracentrifugation and gel permeation chromatography produced a purified protein. LTP is a very high density lipoprotein with a particle M_r > 500,000. Sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed that LTP is comprised of two apoproteins: apoLTP-I (M_r ~ 320,000) and apoLTP-II (M_r ~ 85,000). LTP may have a physiological role in altering the lipid content and composition of the major hemolymph lipoprotein, lipophorin.

The transport of lipophilic biomolecules through insect hemolymph is mediated by lipophorin, the major hemolymph lipoprotein (1,2). *Manduca sexta* lipophorin is a high density lipoprotein with a broad range of lipid components including diacylglycerol, phospholipid, cholesterol, hydrocarbon and small amounts of other neutral lipids and free fatty acids (3). The mechanism of lipid transport by lipophorin is unknown but accumulating evidence suggests that the lipid content of lipophorin can be altered without destruction of a basic lipoprotein matrix structure. *In vivo* studies (4) on locust lipophorin have indicated that lipophorin lipid turns over at a faster rate than the protein moiety. During *M. sexta* larval-pupal development, in the absence of significant lipophorin protein synthesis, at least four lipophorin forms, distinct in lipid content and composition, occur in hemolymph (3). A hemolymph factor, capable of catalyzing net lipid transfer

Abbreviations: LTP = lipid transfer protein; HDLp-A = high density lipophorin-adult; HDLp-W2 = high density lipophorin-wanderer 2; SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis; PBS = phosphate buffered saline.

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between lipophorins of different densities has been shown to exist in M. sexta (5). We report here the isolation and preliminary characterization of M. sexta lipid transfer protein (LTP) and compare its structural features with those of other hemolymph lipoproteins.

MATERIALS AND METHODS

Animals: Manduca sexta eggs were generously supplied by Drs. J. Buckner and J. P. Reinecke, United States Department of Agriculture, Fargo, ND. Animals were reared as previously described (3).

Purification of LTP: One hundred late fifth instar M. sexta mixed sex larvae were bled through an incision in the second proleg. Hemolymph was placed in 0.10 M sodium phosphate pH 7.0, 0.15 M NaCl (PBS) containing 50 mM glutathione and 1 mM diisopropylphosphorofluoridate. Following a low speed centrifugation (3,000 g at 4°C for 10 min) to remove hemocytes, the hemolymph was brought to a density of 1.31 g/ml with solid KBr (20 ml final volume), overlayered with 0.9 percent NaCl and centrifuged in a VTi50 rotor at 50,000 rpm for 16 h at 10°C (6). The d > 1.21 g/ml fractions (lipophorin-deficient hemolymph) were collected and dialyzed against PBS. Solid ammonium sulfate was added to the sample and at 45 percent saturation the material was centrifuged at 20,000 g for 20 min. The pellet obtained was dissolved in 10 ml PBS and applied to a 2.5 x 86 cm column of Bio-Gel A 1.5 equilibrated in PBS. The column was eluted at a flow rate of 12 ml/h with collection of 4 ml fractions. LTP containing fractions were pooled and concentrated by ultrafiltration (Amicon YM30 membrane). The concentrated sample was then brought to a density of 1.31 with solid KBr (20 ml final volume), placed in a 39 ml Beckman Quick Seal tube and overlayered with a solution (KBr in PBS) of d = 1.23 g/ml and centrifuged in a VTi50 rotor for 16 h at 50,000 rpm and 4°C as described by Haunerland and Bowers (7). Following centrifugation, purified LTP was collected, dialyzed into PBS and stored under N_2 atmosphere.

Lipid Transfer Assay: The assay of net lipid transfer was performed as previously described (5) except that 1 mg each of donor and acceptor lipophorin were used. In this study the donor lipophorin employed was high density lipophorin-adult (HDLp-A, d = 1.078) while the acceptor lipophorin was high density lipophorin-wanderer 2 (HDLp-W2, d = 1.18). HDLp-W2 and HDLp-A were isolated as previously described (3,5).

Other Analyses: Methods for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and fluorescent lectin staining have been reported elsewhere (8). The equilibrium density of LTP was determined after a 16 h density gradient ultracentrifugation, fractionation of the tube contents and measurement of the refractive index of KBr in LTP containing fractions. In addition to commercial (Bio-Rad) molecular weight standards for SDS-PAGE, chemically crosslinked M. sexta arylphorin (9) was employed.

RESULTS AND DISCUSSION

An in vitro assay of facilitated net lipid transfer between lipoproteins of different densities was employed to monitor the purification of M. sexta LTP (Fig. 1). In the absence of LTP no change in donor (HDLp-A) or acceptor (HDLp-W2) lipophorin density occurred following in vitro incubation while LTP

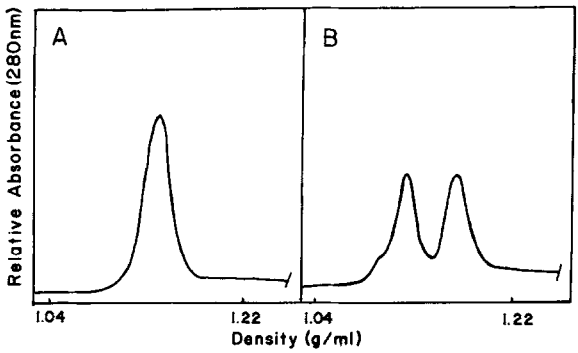

Figure 1: In vitro lipid transfer assay. 10 μg LTP (A) or PBS (B) was added to 1 mg each of HDLp-A and HDLp-W2 (final volume = 1.0 ml) and incubated for 1 h at 26°C. The samples were then centrifuged for 4 h at 50,000 rpm and 10°C. After centrifugation the tube contents were fractionated from the top with continuous monitoring of absorbance (280 nm).

addition to the incubation solution caused production of a single intermediate density lipophorin particle. LTP was purified from lipophorin-deficient hemolymph by taking advantage of two of its unique physical properties, size and density. Figure 2 shows the progress of LTP purification

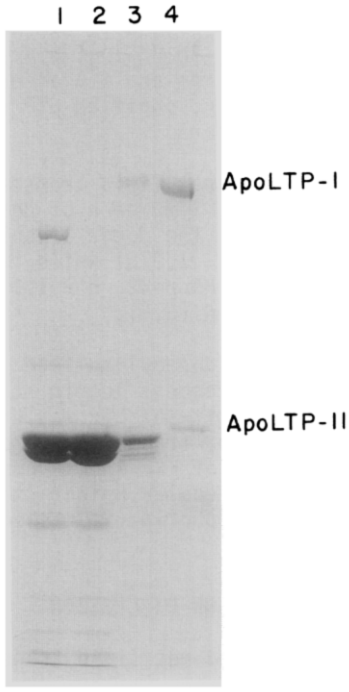

Figure 2: SDS-PAGE analysis of LTP purity after successive purification steps. A 4-10 percent polyacrylamide gradient slab was electrophoresed at 30 mA for 3.5 h. Lane 1, fifth instar larval hemolymph; lane 2, lipophorin deficient hemolymph; lane 3, after gel permeation chromatography; and lane 4, after second density gradient ultracentrifugation step.

after each step. LTP eluted ahead of most other hemolymph proteins when subjected to gel permeation chromatography and was completely separated from remaining contaminants by a density gradient ultracentrifugation step which results in flotation of very high density lipoproteins. Isolated LTP was determined to have a density of 1.23 g/ml which suggests it may contain 10-15 percent lipids by weight. The protein moiety of LTP is composed of two apoproteins, apoLTP-I and apoLTP-II. The molecular weight of LTP apoproteins was determined by SDS-PAGE to be ~320,000 and 85,000, respectively. Scanning densitometry of LTP apoproteins separated by SDS-PAGE revealed a 3:1 ratio of staining intensity suggesting a 1:1 apoprotein ratio in the native particle. Both apoproteins possess covalently bound mannose containing oligosaccharide residues, as judged by fluorescent concanavalin A binding to apoproteins separated by SDS-PAGE. A particle molecular weight estimate of >500,000 is indicated, based on gel filtration data.

A comparison of the apoprotein components of LTP with that of M. sexta HDLp-W2 and M. sexta vitellogenin (10) (Fig. 3) reveals interesting similarities in these insect lipoprotein structures. All three lipoprotein particles contain a high molecular weight apoprotein and a lower molecular weight apoprotein in addition to their respective lipid components. Thus LTP, lipophorin and vitellogenin may represent members of a family of structurally related but functionally and immunologically distinct lipoproteins. Whereas lipophorin functions in the transport of ingested and stored lipid to tissues of utilization and vitellogenin supplies nutrients to the developing egg, LTP appears to play a role in facilitating lipid transfer perhaps between lipoproteins or lipoproteins and membranes.

This paper describes for the first time isolation of an insect hemolymph lipid transfer protein. Several studies (2,3,11) on the hemolymph lipoprotein lipophorin have indicated that lipophorin functions as a reusable lipid shuttle. However, the mechanism of lipid transport without protein turnover has not been elucidated. We have demonstrated that LTP efficiently catalyzes net lipid transfer among lipoproteins and apparently is the major source of

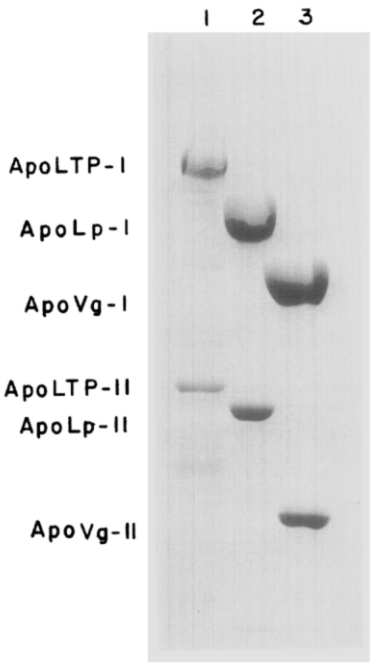

Figure 3: SDS-PAGE of M. sexta hemolymph lipoproteins. (1) LTP, (2) lipophorin (HDLp-W2), (3) vitellogenin. Apolp-I and II: Apolipophorin I and II; ApoVg-I and II: Apovitellogenin I and II, respectively.

lipid transfer activity previously observed in lipophorin-deficient hemolymph (5). The possible physiological role of LTP in mediating lipid mobilization from fat body to lipophorin or from lipophorin to tissues of lipid utilization remains to be explored. The availability of purified LTP will allow us to address important questions regarding the physiological role of LTP in the adipokinetic hormone stimulated diacylglycerol mobilization events associated with flight metabolism (12). Complete characterization of the physical, chemical and immunological properties of LTP is presently in progress and should offer interesting comparisons with mammalian plasma lipid transfer proteins, especially human lipid transfer complex which has been reported to exist as a heterogeneous lipoprotein (13).

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