Isolation and Characterization of a Lipoprotein Receptor from the Fat Body of an Insect, *Manduca sexta*

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A lipoprotein receptor has been purified from the fat body of *Manduca sexta* larvae. The purification involves solubilization of membrane proteins in detergent, DEAE-, and hydroxyapatite chromatography, affinity chromatography on a concanavalin A column, and affinity chromatography on a lipoprotein-Sepharose column. An overall purification of 220-fold from the solubilized membranes was achieved. The receptor has an apparent molecular mass of 120 kDa. The receptor has an absolute requirement for Ca2+ and is inhibited by Suramin. The pH optimum of the receptor is 6.5, which is near the pH of the hemolymph. Binding data indicate a single high affinity binding site with a Kd = 4.1 × 10^{-4} M as measured with the lipoprotein isolated from larval hemolymph. The major neutral lipid carried by insect lipoproteins is diacylglycerol, and it was shown that the affinity of the receptor for lipoprotein ligands correlates with their diacylglycerol content. It is proposed that the decrease in affinity of the receptor for lipoproteins depleted of diacylglycerol plays a key role in facilitating the transport of diacylglycerol from the midgut to the fat body during the larval feeding period. The insect receptor has some properties which are similar to those of vertebrate lipoprotein receptors, viz. molecular weight, requirement for Ca2+, and inhibition by Suramin. However, the insect receptor does not bind human low density lipoprotein.

In mammalian systems, the LDL1 (apolipoprotein B/E) receptor has been extensively characterized (Brown and Goldstein, 1986), but the mechanisms by which other plasma lipoproteins, e.g. acetyl-LDL, β-VLDL, chylomicron remnants, and HDL, transfer their lipids to and from cells remain obscure. In some cases, it is clear that an internalizing receptor is involved, e.g. chylomicron remnants (Mahley and Innerarity, 1983). However, in the case of HDL, the situation is less clear. While there is evidence for a HDL receptor (Oram et al., 1983), its function has been questioned (Karlin et al., 1987). Irrespective of the characterization of a HDL receptor, it is evident that HDL can deliver cholesterol to some cells via a receptor-independent pathway (see for example Pitman et al., 1987; Azhar et al., 1988; Rinninger and Pitman, 1989).

In insect model systems, the mechanism of transfer of lipid from lipoproteins to cells has received scant attention. It is known that during flight lipid is mobilized from its storage site, the fat body, transported through the hemolymph in the form of diacylglycerol (the major transported lipid in insect lipoproteins) associated with lipoprotein, and delivered to the flight muscle, where it is used for energy production. This lipid transport occurs without synthesis of new molecules of apolipoprotein and without destruction of existing apolipoprotein molecules (see Shapiro et al., 1988 for a review). In addition, we have previously shown that when the fat body of a feeding *Manduca sexta* larva was incubated in vitro with the hemolymph lipoprotein, high density lipoprotein (HDLp), there was nearly complete transfer of diacylglycerol to the fat body without detectable internalization of lipoprotein apolipoprotein (Tsuchida and Wells, 1988). These observations are consistent with the suggestion that in insects lipid, at least diacylglycerol, can move between lipoprotein and cells without internalization of the lipoprotein particle. However, it is not clear whether such systems require a membrane receptor for the lipoprotein in order to facilitate lipid transfer. We have therefore isolated and characterized a lipoprotein receptor from larval fat body as a first step in understanding lipid transport to cells in insects.

**MATERIALS AND METHODS**

Lipoprotein Isolation and Iodination—Hemolymph was prepared by the flush method (Chino et al., 1987). Lipoproteins were isolated by density gradient centrifugation following the procedure of Prasad et al. (1986a) for high density lipoprotein-larval (HDLp-L), high density lipoprotein-wanderer 1 (HDLp-W1), and high density lipoprotein-wanderer 2 (HDLp-W2). Egg lipoprotein (VHDLp-E) was isolated from eggs removed from day 2 adults using a modification of the method of Kawooya et al. (1988). Eggs were homogenized in 50 mM phosphate buffer, pH 7.0, containing 0.15 M NaCl, 0.1 M diisopropyl fluorophosphate, 5 mM benzamidine, 5 mM EDTA, and a protease inhibitor mixture (Prasad et al., 1986b) using a Polytron homogenizer and 10 ml of buffer/g of eggs. The homogenate was centrifuged two times at 100,000 × g for 30 min. The clear supernatant was adjusted to a density of 1.31 g/ml with solid KBr and a volume of 20 ml, placed in a 39-ml Beckman Quick Seal centrifuge tube, and overlayed with a KBr solution of density 1.21 g/ml. The solution was centrifuged for 16 h at 50,000 rpm in a VTI 50 rotor at 5 °C. The top 5 ml were removed from the tube and centrifuged again under the same conditions. The very high density lipoprotein present in the hemolymph of insects on a fat-free diet (VHDLp-FF) was isolated as described by Prasad et al. (1986b). Human LDL was kindly provided by Dr. Donald McNamara (University of Arizona). Lipoproteins were iodinated with sodium [125I]iodide (17.4 Ci/mg, DuPont-New England Nuclear, Wilmington, DE) using IODO-GEN (Pierce) following the manufacturer’s instructions.
Assay of Lipoprotein Receptor—The receptor solution in various buffers was combined with a suspension of hen's egg yolk phosphatidylcholine (Avanti Polar Lipids Inc., Pelham, AL) and buffer to achieve a final Triton X-100 concentration of 0.2% and a phospholipid concentration of 0.1-0.2 mg/ml and 700 mM NaCl and 2 mM CaCl2 (Si). The mixture of ice-cold acetone was added, and, after mixing, the precipitate was immediately collected by centrifugation at 20,000 × g for 20 min. The pellet was resuspended in 100-500 μl of assay buffer (5 mM Tris-HCl, 10 mM MOPS, 150 mM NaCl, and 2 mM CaCl2, 0.25% bovine serum albumin, pH 7.0) by aspiration through a 22-gauge needle. The suspension was incubated with the indicated amount of 125I-HDLp-L and unlabeled HDLp-L or other lipoproteins at room temperature (25 °C) for 1 h. Aliquots (200 μl) were removed and applied to 25-mm Millipore filters, type GVWP, with a 0.22-μm pore size (Millipore Corp.). Unbound lipoprotein was removed by filtration and by washing the filters seven times with cold buffer (1 ml/wash). The filters were counted in a γ-counter.

Specific binding was determined using a 100-fold excess of unlabeled HDLp-L. High affinity binding was calculated by subtracting the value for nonspecific binding from the value for total binding.

Preparation and Solubilization of Fat Body Membranes—Fat body membranes were isolated from 1-3 fifth instar. M. sexta larvae was homogenized in ice-cold buffer (20 mM Tris-HCl, 150 mM NaCl, 2 mM CaCl2, pH 7.4) containing a protease inhibitor mixture (Prasad et al., 1986b) using 5 ml/g tissue and a Polytron homogenizer. The homogenate was centrifuged at 1,000 × g for 10 min, and the supernatant was filtered through cheesecloth and centrifuged at 800 × g for 10 min. The membranes were then pelleted by centrifugation at 100,000 × g for 1 h and resuspended in 20 mM Tris-HCl, 150 mM NaCl, 2 mM CaCl2, 0.1 mM PMSF, pH 7.4, at a concentration of 10 mg of protein/ml. This suspension was sonicated for 15 s (Branson Sonifier 200, microprobe setting 3) and diluted with an equal volume of 2% Triton X-100 in the same buffer. After mixing for 1 h at 4 °C, insoluble material was removed by centrifugation at 100,000 × g for 1 h.

Purification of the Receptor—All operations were carried out at 4 °C. The detergent-solubilized membranes were applied to a column (2.5 × 8 cm) of DEAE-Trisacryl M (IBF, France) equilibrated with 20 mM Tris-HCl, 50 mM NaCl, 2 mM CaCl2, 0.1 mM PMSF, pH 7.4, containing 1% Triton X-100. The column was eluted with 300 ml of a linear gradient of 50-600 mM NaCl in the same buffer at a flow rate of 5 ml/h, and 5-ml fractions were collected. The DEAE-fractons containing receptor activity were pooled and dialyzed overnight against 15 mM potassium phosphate buffer, pH 6.5, containing 150 mM NaCl and 1% Triton X-100. The solution was applied to a column (2.5 × 8 cm) of Bio-Gel HT hydroxypatite (Bio-Rad), which had been equilibrated with dialysis buffer. After washing the column with 100 ml of the same buffer, the column was eluted with a 200-ml linear gradient of 15-500 mM potassium phosphate, pH 6.5, containing 1% Triton X-100 at a flow rate of 15 ml/h, and 2.5-ml fractions were collected. The active fractions from the hydroxypatite chromatography were pooled and dialyzed overnight against 5 mM Tris, 10 mM MOPS, 150 mM NaCl, 2 mM CaCl2, 1 mM MnCl2, 1 mM MgCl2, 0.02% NaN3, 0.1 mM PMSF, pH 7.0. This solution was then applied to a column (1.5 × 7 cm) containing Affi-Gel-ConA (Bio-Rad) equilibrated in dialysis buffer. After thoroughly washing the column with the same buffer, the column was eluted with 0.2 M α-D-methyllnopyranoside in the same buffer. The fractions containing the receptor were diluted with 4 volumes of the same buffer without Triton X-100 and then dialyzed overnight against 5 mM Tris, 10 mM MOPS, 150 mM NaCl, 1 mM MgCl2, 2 mM CaCl2, 0.1 mM PMSF, 0.02% NaN3, pH 6.5, containing 0.2% Triton X-100. After centrifugation at 100,000 × g for 1 h, the clear supernatant was mixed with 10 ml of an affinity gel prepared by coupling HDLp-L to CNBr-activated Sepharose 4B (10 mg of HDLp-L per g of dry gel). The suspension was gently stirred at 4 °C overnight and transferred to a column, and the column was washed with 50 ml of 0.05 mM Tris, 10 mM MOPS, 150 mM NaCl, 0.1 mM PMSF, 0.02% NaN3, pH 7, containing 0.2% Triton X-100. The elution was collected by recycling 20 ml of 5 mM Tris, 10 mM MOPS, 150 mM NaCl, 5 mM Suramin (Mobay Chemical Corp., New York), 5 mM EDTA, 0.1 mM PMSF, 0.02% NaN3, pH 7, containing 0.2% Triton X-100 at room temperature for 4 h. The eluate was dialyzed against 20 mM Tris-HCl, pH 7.4, 50 mM NaCl, 2 mM CaCl2, 0.02% NaN3, 0.1 mM PMSF containing 0.2% Triton X-100 and then applied to a column (2 × 3 cm) of DEAE-Trisacryl M, which had been equilibrated in the same buffer. After washing thoroughly with the same buffer, the receptor was eluted with 300-500 μl of 5 mM Tris-HCl, pH 7.4, 350 mM NaCl, 2 mM CaCl2, 0.02% NaN3, 0.1 mM PMSF containing 0.2% Triton X-100. This step was used to concentrate the receptor and remove material eluted from the HDLp-L affinity column. The active fractions were pooled and concentrated by pressure dialysis using a Diaflo Ultrafiltration membrane YM-30 (Amicon Corp., Danvers, MA).

Polyacrylamide Gel Electrophoresis and Ligand Blotting—Electrophoresis in the presence of SDS was performed on 4-15% linear gradients of polyacrylamide according to the method of Laemelli (1970). Samples from fat body membranes were incubated at 37 °C for 10 min in SDS sample buffer without a sulfhydryl reducing reagent. Electrophoresis was carried out at 4 °C at 30 mA/slab gel for 3 h, and gels were calibrated with molecular weight standards (Sigma). Gels were stained with Coomassie Brilliant Blue R. Proteins were transferred from SDS-slab gels to nitrocellulose paper by the method of Towbin et al. (1979). After electrophoretic transfer, the nitrocellulose paper was incubated with 2% non-fat dry milk, 1% bovine serum albumin, and 1% hemoglobin in 6 mM Tris, 10 mM MOPS, 150 mM NaCl, and 2 mM CaCl2 for 1 h at 25 °C. After the blocking reaction, the paper was incubated with 50 μg/ml 125I-HDLp-L (263.9 cpm/ng) and various amounts of unlabeled HDLp-L. Autoradiography was carried out by exposing the dried paper to Kodak X-Omat film for 24 h at −70 °C.

Protein Determination—The concentration of protein was measured by the method of Lowry as modified by Bensadoun and Weinstein (1976). In the case of samples containing Triton X-100, the sample was diluted with water to a detergent concentration of 0.1% before assay, and varying concentrations of bovine serum albumin in 0.1% Triton X-100 were used as standards.

RESULTS

Evidence for a Lipoprotein Receptor in Fat Body Membranes—Preliminary studies were carried out to determine whether fat body membranes would bind lipophorin and whether the binding activity could be attributed to a membrane protein. Fig. 1 shows the time dependence of specific and nonspecific lipophorin binding to fat body membranes and demonstrates that equilibrium is achieved after 1 h. Fig. 2 presents the effect of membrane protein concentration on the amount of lipoprotein bound and establishes that receptor binding activity can be measured using 20-100 μg of membrane protein. These results clearly establish that fat body membranes contain a high affinity, specific binding site for lipophorin. Table I shows the effect of protease and heat treatments on lipophorin binding by fat body membranes and establishes that the binding activity is due to a protein in the membrane.
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**FIG. 2.** Relationship between fat body membrane concentration and lipoprotein binding. Each tube contained 30 ug/ml 125I-HDLp-L (118 cpm/ng of protein) and the indicated amount of fat body membrane protein in the absence (C) or presence (A) of 1 mg/ml unlabeled HDLp-L. The total amount of labeled HDLp-L bound to membranes was determined. Circles show specific binding of HDLp-L. These data are for a typical experiment run in triplicate. The standard error of determination was less than the size of symbols used in the figure.

**TABLE I**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HDLp-L bound</th>
<th>Percent</th>
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<tbody>
<tr>
<td>None</td>
<td>5.8</td>
<td>100</td>
</tr>
<tr>
<td>Trypsin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 pg/ml, 10 min</td>
<td>1.4</td>
<td>24.1</td>
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<tr>
<td>10 pg/ml, 10 min</td>
<td>0.3</td>
<td>5.2</td>
</tr>
<tr>
<td>5 pg/ml, 30 min</td>
<td>0.3</td>
<td>5.2</td>
</tr>
<tr>
<td>10 pg/ml, 30 min</td>
<td>0.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Trypsin (5 pg/ml) + soybean trypsin inhibitor (20 pg/ml), 30 min</td>
<td>5.2</td>
<td>89.6</td>
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Specificity of the Ligand Blotting Assay—The proteins in the active fraction from the DEAE-step in the purification scheme (see below) were analyzed by the ligand blotting procedure. As can be seen from Fig. 3, this procedure detected a single band with an apparent molecular mass of 120 kDa, which corresponded to a minor protein band in the Coomassie-stained gel. On this basis, we concluded that the ligand blotting assay shows the necessary specificity to detect the lipophorin receptor.

**FIG. 3.** Specificity of the ligand blotting assay. Panel A, SDS-PAGE and Coomassie Brilliant Blue staining of fractions from the DEAE-column (see Fig. 4). a, molecular weight standards; b and c, DEAE-flow through fractions; d, lipophorin binding fraction. Panel B, lipophorin binding fraction, 50 pg (lane e) or 100 pg (lane f) of protein were separated by SDS-PAGE and transferred to nitrocellulose. The nitrocellulose was incubated with 125I-HDLP-L (20 pg/ml), and, after washing, the blot was exposed to x-ray film for 1 day at -80 °C.

**FIG. 4.** DEAE-column chromatography of the fat body lipophorin receptor. Detergent-solubilized fat body membranes were applied to a column (2.5 x 15 cm) of DEAE-Trisacryl M equilibrated with 20 mM Tris-HCl, 50 mM NaCl, 2 mM CaCl2, 0.1 mM PMSF, pH 7.4, containing 1% Triton X-100. The column was eluted with 300 ml of a linear gradient of 50–600 mM NaCl in the same buffer at a flow rate of 25 ml/h, and 5-ml fractions were collected. The open circles represent protein, and the closed circles receptor binding activity.

Specificity of the Ligand Blotting Assay—The proteins in the active fraction from the DEAE-step in the purification scheme (see below) were analyzed by the ligand blotting procedure. As can be seen from Fig. 3, this procedure detected a single band with an apparent molecular mass of 120 kDa, which corresponded to a minor protein band in the Coomassie-stained gel. On this basis, we concluded that the ligand blotting assay shows the necessary specificity to detect the lipophorin receptor.

Purification of the Receptor—The receptor was purified from detergent-solubilized fat body membranes by a combination of DEAE-Trisacryl M chromatography, hydroxyapatite chromatography, and affinity chromatography. In preliminary experiments, we found no difference in recovery of receptor activity whether we used Triton X-100 or octyl glucoside as the detergent for membrane extraction, and therefore we used Triton X-100 in all our procedures. Fig. 4 shows the result of a typical run on the DEAE-column.
Receptor activity was eluted as a single peak at a NaCl concentration of about 300 mM. The elution profile of the receptor from the hydroxyapatite column is shown in Fig. 5. At this stage, we attempted a final purification of the receptor by ligand affinity chromatography using a HDLp-L-Sepharose column; however, we did not obtain pure receptor. Finally, it was determined that the use of a ConA affinity column prior to the lipophorin affinity column gave pure material. Table II summarizes the purification scheme and shows that the receptor was purified approximately 220-fold with an overall yield of about 6%. SDS-PAGE of the purified receptor under nonreducing conditions shows a single band with an apparent molecular mass of 120,000 daltons (Fig. 6A).

Characterization of the Receptor—Due to the very small amount of pure receptor which can be obtained, we chose to characterize the properties of the receptor using the material after the first DEAE-chromatography step. Fig. 7 presents the concentration dependence of HDLp-L binding to the receptor and the Scatchard plot of specific binding data. The binding data indicate a single high affinity binding site with a $K_d = 4.1 \pm 0.19 \times 10^{-9}$ M. Fig. 8 shows the pH dependence of lipoprotein protein binding to the receptor, which has an optimum around pH 6.5 (we determined that the pH of larval hemolymph is about pH 6.7). As shown in Fig. 9, EDTA inhibits binding of HDLp-L to the receptor, and this inhibition can be reversed by addition of Ca$^{2+}$. Mg$^{2+}$, at saturating levels (2.5 mM), has only 20% of the activity of Ca$^{2+}$ in stimulating receptor binding.

It has been shown that Suramin, a polysulfated polycyclic hydrocarbon, inhibits binding of LDL to the human LDL receptor, and LDL and VLDL to the chicken oocyte LDL/VLDL receptor, and promotes dissociation of lipoproteins from their receptors (Schneider et al., 1982; George et al., 1987). Therefore, the effect of Suramin on the fat body

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**Table II**

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein</th>
<th>Specific binding</th>
<th>Purification</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubilized membranes</td>
<td>116</td>
<td>0.47</td>
<td>-fold</td>
<td>100</td>
</tr>
<tr>
<td>DEAE-column</td>
<td>22.4</td>
<td>0.93</td>
<td>2.0</td>
<td>38.2</td>
</tr>
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<td>Hydroxyapatite column</td>
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<td>3.4</td>
<td>7.2</td>
<td>27.5</td>
</tr>
<tr>
<td>Aff-ConA column</td>
<td>0.85</td>
<td>16.4</td>
<td>34.9</td>
<td>25.5</td>
</tr>
<tr>
<td>HDLp-L affinity column</td>
<td>0.001</td>
<td>102.8</td>
<td>218.7</td>
<td>5.9</td>
</tr>
</tbody>
</table>

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**Fig. 5.** Hydroxyapatite chromatography of the fat body lipoprotein receptor. The DEAE-fractions containing receptor activity were pooled and dialyzed overnight against 15 mM potassium phosphate buffer, pH 6.5, containing 150 mM NaCl and 1% Triton X-100. The solution was applied to a column (2.5 x 8 cm) of Bio-Gel HT hydroxyapatite, which has been equilibrated with the dialysis buffer. After washing the column with 100 ml of the same buffer, the column was eluted with 200 ml of linear gradient of 15-500 mM potassium phosphate, pH 6.5, containing 1% Triton X-100 at a flow rate of 15 ml/h, and 2.5-ml fractions were collected. The open circles represent protein, and the closed circles receptor binding activity.
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FIG. 8. Effect of pH on the binding of HDLp-L to its receptor. Each tube contained 30 pg/ml '*'I-HDLp-L (215 cpm/ng of protein) and 20 pg/ml DEAE-purified receptor. Tris maleate buffer (20 mM) was used in the pH range 5.5 to 7.0 (open circles), and Tris-HCL buffer (20 mM) was used in the pH range 7.4 to 8.5 (closed circles). These data are for a typical experiment run in triplicate. The standard error of determination was less than the size of symbols used in the figure.

Fig. 10. Effect of Suramin on the binding of HDLp-L to its receptor. Left panel, effect of Suramin on formation of the HDLp-L-receptor complex. Each tube (250 µl) contained 20 µg/ml DEAE-purified receptor and 20 µg/ml '*'I-HDLp-L (112 cpm/ng of protein) and the indicated concentration of Suramin. After incubation for 1 h, the amount of HDLp-L bound to the receptor was determined. Right panel, effect of Suramin on dissociation of the HDLp-L-receptor complex. Each tube (190 µl) contained 20 µg/ml DEAE-purified receptor and 20 µg/ml '*'I-HDLp-L (112 cpm/ng of protein). After incubation for 30, 60, or 120 min, 10 µl of 50 mM Suramin in water was added to each tube, and the incubation continued for either 1, 10, or 30 min. At these times, the amount of HDLp-L bound to the receptor was determined. Nonspecific binding (A) was determined in the presence of 1 mg/ml unlabeled HDLp-L. These data are for a typical experiment run in triplicate. The standard error of determination was less than the size of symbols used in the figure.

receptor was tested, and Fig. 10 shows that Suramin both inhibits binding of HDLp-L to the fat body receptor and promotes dissociation of HDLp-L from the receptor. The ability of Suramin to cause dissociation of the HDLp-L-receptor complex was used to advantage in the purification of the receptor.

Fig. 11 shows that the binding of HDLp-L to its receptor is not inhibited by the proteins present in lipophorin-free hemolymph, or by arylphorin, a major larval hemolymph protein, or by vitellogenin, a major hemolymph protein in adult females which is taken into the developing egg by a receptor-mediated process. On this basis, we conclude that the receptor is specific for lipoproteins. Fig. 12 shows the specificity of binding of various lipoproteins to the larval fat body receptor as deduced from the ability of the lipoproteins to compete with the binding of '*'I-HDLp-L to the receptor. The insect lipoproteins were chosen in order to determine the effect of diacylglycerol content of the lipoprotein on receptor binding affinity. The order of affinity decreases in the order HDLp-L > HDLp-W1 > HDLp-W2 > VHDLp-FF > VHDLp-E, with little or no binding exhibited by human LDL. When the concentration of each lipoprotein required to inhibit '*'I-HDLp-L binding by 50% was plotted against the diacylglycerol content of the lipoprotein, as reflected in the molar ratio of diacylglycerol/phospholipid, a linear relationship was found, which shows that in this series of lipoproteins the
higher the proportion of diacylglycerol the higher the affinity for the receptor (Fig. 12B). It should be noted that the insect lipoproteins shown in Fig. 12B all have the same apolipoprotein composition and differ only in lipid content and composition. Thus, in spite of the fact that some of these lipoproteins are not physiologically relevant ligands for the receptor in larval fat body, it is clear that diacylglycerol content has an important effect on the affinity of the receptor for its lipoprotein ligand.

**DISCUSSION**

The purification scheme developed to purify the lipophorin receptor from the fat body is rather similar to that used to purify the human LDL receptor (Schneider et al., 1982). In fact, the insect fat body receptor is similar in some respects to the human LDL receptor and the chicken oocyte LDL/VLDL receptor (Schneider et al., 1982; Daniel et al., 1983; George et al., 1987). Thus, all three receptors have similar molecular masses as determined by electrophoresis under nonreducing conditions followed by ligand blotting: human receptor = 130 kDa, chicken oocyte receptor = 95 kDa, M. sexta receptor = 120 kDa. All three receptors require Ca\(^{2+}\) and are inhibited by Suramin, a property they share with the apolipoprotein E receptor (Hui et al., 1986) and the locust vitellogenin receptor (Röhrkasten and Ferenz, 1987). Based on its binding to a ConA column, we assume the fat body receptor is a glycoprotein with high mannose type oligosaccharide chains, a property it also shares with the human LDL receptor.

The K\(_d\) of 4.1 × 10\(^{-8}\) M (about 25 μg/ml) is well below the concentration of lipophorin found in larval hemolymph, 4–5 mg/ml (Prasad et al., 1987), which means the receptor is saturated under physiological conditions. The K\(_d\) is comparable to that of the human LDL receptor and the chicken oocyte LDL/VLDL receptors. However, unlike the vertebrate receptors which show considerable cross-species ligand binding capacity (George et al., 1987), the insect receptor did not bind human LDL.

The mammalian LDL receptor serves to internalize the entire lipoprotein ligand (Brown and Goldstein, 1980). In contrast, in in vitro studies of lipid transport to the larval fat body only diacylglycerol was transferred from HDLp-L to the fat body, and a diacylglycerol-depleted very high density lipophorin accumulated in the incubation medium (Taschuda and Wells, 1988). The mechanism whereby diacylglycerol is selectively transferred from lipophorin to the fat body is unknown at present. It is possible that the lipid transfer particle described by Ryan et al. (1988) might be involved; however, recent studies by van Heusden and Law (1989) using adult M. sexta fat body suggest that the lipid transfer particle is not required for transfer of lipid from lipophorin to fat body but only for the reverse process.

Regardless of the details of the lipid transfer mechanism, the relationship between receptor affinity and the diacylglycerol content of the lipophorin ligand shows that the receptor can distinguish between a diacylglycerol-rich and a diacylglycerol-depleted lipoprotein. Thus, as diacylglycerol is lost from HDLp-L, the lipoprotein would be converted to a diacylglycerol-depleted very high density lipophorin which would dissociate from the receptor and be replaced by HDLp-L, due to both the higher affinity of HDLp-L for the receptor and its higher concentration in hemolymph. The origin of the change in affinity for the receptor caused by the loss of diacylglycerol is unknown at present. It may result from a conformational change in the apolipoproteins caused by the reduction in lipoprotein surface area which would result from the decreased core lipid volume accompanying loss of diacylglycerol. For example, since diacylglycerol accounts for about 50% of the core volume of HDLp-L (Shapiro et al., 1988), the reduction in total surface area of the lipoprotein following complete loss of diacylglycerol would amount to approximately 10%, which may be sufficient to cause a significant reorientation of the apolipoproteins at the surface.

It has been proposed that lipophorin cycles between midgut and fat body in order to deliver diacylglycerol derived from dietary fat to the fat body for storage. For such a cycle to function, the diacylglycerol-depleted very high density lipophorin produced after diacylglycerol delivery to the fat body should bind to the midgut and pick up additional diacylglycerol and in the process be converted to HDLp-L. Such a cycle would require the presence of a receptor in midgut with higher affinity for diacylglycerol-depleted very high density lipophorin than for HDLp-L.

In summary, we have described the purification and properties of a lipoprotein receptor from the fat body of M. sexta. The insect receptor has several properties which are similar to those of vertebrate lipoprotein receptors. However, the insect receptor does not bind vertebrate lipoproteins. The properties of the fat body receptor suggest a mechanism of transport of lipid from the insect midgut to the fat body which depends on changes in the receptor binding properties of the lipoprotein's apolipoproteins as the content of the diacylglycerol in the lipoprotein changes.

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