Chylomicron protein content and the rate of lipoprotein lipase activity

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Abstract Chylomicrons isolated from rat intestinal lymph were incubated with plasma. Protein transfer to chylomicrons, reaction rate with purified lipoprotein lipase, and content of lipase cofactor were determined. While the overall protein content of chylomicrons was increased 3–4-fold, and the content of lipase cofactor increased 4-fold, reaction velocity of the activated particles with lipoprotein lipase was increased only 1.3-fold. Maximal rate of hydrolysis was achieved in the presence of much smaller quantities of activator than the lipoprotein particles were capable of binding, and chylomicrons were fully activated for triglyceride hydrolysis in the presence of only 10% plasma for triglyceride concentrations of up to 3 mg/ml. Cofactor protein was not rate-limiting for hydrolysis of triglyceride from chylomicrons. These results are discussed in the light of recent concepts of the regulation of lipoprotein lipase activity.

Supplementary key words cofactor protein · chylomicrons

Chylomicrons entering the plasma from intestinal lymph receive a variety of apoproteins by transfer from the plasma high density lipoprotein fraction, including the protein cofactor of LPL, whose activity forms the major pathway of catabolism of plasma triglyceride (1, 2). These proteins are supplied to the chylomicrons from a reservoir located primarily in the plasma high density lipoprotein fraction (3). The activated chylomicron particles form an effective substrate for LPL both in vitro and at the vascular surface of perfused tissues such as the heart (4). In the course of LPL-catalyzed hydrolysis of chylomicron triglyceride, these proteins are transferred back and the generated "remnant" product of enzyme activity has been shown to be depleted of these migrating protein species (5, 6). In consequence it has been considered that the rate of LPL activity with natural lipoprotein substrates might be limited by the content of LPL cofactor protein (7). The aim of the present study was to determine the role of chylomicron protein in the activation process of lymph chylomicrons.

MATERIALS AND METHODS

Preparation of lymph chylomicrons

Lymph was collected from male Sprague-Dawley rats (300–350 g body wt) who carried an outflow cannula in the mesenteric lymph duct and an inflow cannula into the duodenal lumen, through which was passed triglyceride–lecithin 20% emulsion (Intralipid, the generous gift of Vitrum Company, Stockholm, Sweden) (diluted to 20 mg triglyceride/ml with 0.154 M NaCl) at a flow rate of 2 ml/hr. Lymph was collected for 18 hr into ice-cooled tubes. The major chylomicron fraction (S_f 500–4000) was prepared as previously described (4) by sequential preparative ultracentrifugal flotation. Lymph (2 ml) layered under 8 ml of Krebs-Ringer–EDTA[1], pH 7.4, was centrifuged for 10 min at 12,500 rpm in a Spinco 40-rotor (Spinco Div., Beckman Instruments, Palo Alto, Cal.). The floating fraction was removed, then 2 ml of salt solution was layered over the residual chylomicrons, and the lymph was recentrifuged for 30 min at 17,500 rpm. The floating chylomicron layer was collected and recentrifuged under the same conditions.

Preparation of triglyceride-depleted plasma

Rat blood collected into one-tenth volume of 0.2 M sodium citrate was centrifuged to remove cells, then the plasma very low density lipoproteins and chylomicrons were removed by centrifugation for 24 hr at 40,000 rpm in the Spinco 40.3-rotor. The infranatant solution collected by slicing contained the remaining plasma proteins concentrated 2–3-fold. This solution, after dialysis against Krebs-Ringer–EDTA, was

Abbreviations: LPL, lipoprotein lipase; S_f, flotation index at d 1.063 g/cm^3; BSA, bovine serum albumin; HDL, high density lipoprotein; TG, triglyceride.

[1] Krebs-Ringer–EDTA: 0.144 M NaCl, 0.006 M KCl, 0.0015 M $MgSO_4$, 0.0015 M KH_2PO_4, 0.003 M disodium ethylenediaminetetraacetic acid, pH 7.4.

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mixed with lymph chylomicrons (0.3–3.0 mg triglyceride/ml final concentration) and Krebs-Ringer–EDTA to a final plasma concentration (in terms of protein) (8) of 0–100%, in the presence of Krebs-Ringer bicarbonate buffer[2], pH 7.4. Incubation was for 5–30 min at 37°C. Mixtures were then chilled in ice, and 2–4 ml samples were chromatographed at 3–4°C on columns (2 × 50 cm) of Sepharose 2B gel (Pharmacia, Uppsala, Sweden) equilibrated with 0.154 M NaCl. The flow rate was 20–25 ml/hr and chylomicron triglyceride was eluted quantitatively in the column void volume as previously described (9).

Eluted chylomicrons were analyzed for their content of the major lipid classes (triglyceride, phospholipid, and free and esterified cholesterol) and protein as previously described (4). Electrophoresis of tetramethylurea-soluble chylomicron proteins was carried out in 7.5% polyacrylamide gels in the presence of 8 M urea at pH 8.9 after mixing with an equal volume of tetramethylurea (10). Gels were run at 2–3 mA/tube; they were simultaneously fixed and stained with 0.02% Coomassie Blue in acetic acid–methanol–water 10:45:45 (v/v) at 70°C (then destained in acetic acid–methanol–water 5:5:90 (v/v)).

Triglyceride hydrolysis with purified LPL

Lipase was obtained from postheparin plasma of rats injected 5–7 min previously with 100 IU heparin/kg body wt. LPL activity was purified as previously described (11). Specifically, the plasma was complexed with one-fortieth volume of Intralipid triglyceride emulsion, then the lipase–triglyceride complex was isolated by ultracentrifugal flotation in the 30-rotor of the ultracentrifuge for 30 min at 30,000 rpm. The complex was solubilized by mixing with an equal volume of 0.05 M NH_4OH–NH_4Cl buffer containing 0.5% sodium deoxycholate and 0.5 mM potassium linolenate. After removal of floating lipid by centrifugation for 60 min at 40,000 rpm the soluble fraction was delipidated with acetone and diethyl ether.

The precipitate, dried under vacuum, was redissolved in ammonia buffer–0.5 mM linolenate, and LPL protein was complexed with calcium phosphate. After extraction with 0.2 M potassium oxalate the remaining protein (containing LPL with a sp act of 3000–4000 μmoles FFA released/hr/mg protein at pH 8.3, 37°C) was released with 0.05 M sodium citrate solution. The preparation, homogeneous by several physical techniques, has been characterized previously (11). In the present experiments the assay medium contained (per ml) 30 mg of recrystallized BSA, either at pH 8.3 in the presence of 10 mM Tris-HCl buffer, or at physiological pH (7.4) in the presence of Krebs-Ringer–bicarbonate buffer.

Experiments of two kinds were carried out. In studies of the chylomicron content of LPL cofactor protein, dilutions of chylomicron-containing solutions eluted from Sepharose gel following chromatography, or the same solutions delipidated with ethanol and ether redissolved in ammonia buffer–0.5 mM potassium linolenate (11) were assayed at pH 8.3 in the presence of Tris-HCl buffer and 2 mg/ml of synthetic triglyceride dispersion (Intralipid). Activation curves obtained with this system for LPL cofactor activity had the form of substrate–velocity plots (3) with half-maximal activation proportional to the volume of chylomicron solution added. No significant difference was found between half-maximal activation obtained with intact chylomicrons and their total protein content recovered after delipidation. Activator units are presented in terms of [ml of lipoprotein solution (containing 1.0 mg/ml triglyceride, or its derived total apolipoprotein) for half-maximal activation]$^{-1}$. In these studies chylomicrons from mesenteric lymph, prepared in the absence of added plasma proteins, had an activator content of 10–20 units/mg triglyceride, i.e., half-maximal activation was obtained with 0.05–0.1 ml of lipoprotein solution/ml assay medium.

In the second series of experiments, carried out with the same solutions of original and activated chylomicrons used in the first studies, chylomicrons (diluted from the Sepharose eluates after incubation) were the substrate for the activity of purified lipase in the presence of either Tris-HCl (pH 8.3) or Krebs-Ringer–bicarbonate (pH 7.4).

In all experiments lipase activity, linear for at least 10 min at 37°C, was determined titrimetrically as the rate of release of unesterified fatty acid from triglyceride (11). Reaction of 1 ml assays was stopped by addition of 5 ml of acidified heptane–isopropanol, then the phases were separated by addition of 2.5 ml of heptane and the same volume of water. Fatty acid was determined in samples of the heptane phase using a Radiometer ABU-11 autoburette.

RESULTS

Incubation of lymphatic chylomicrons with increasing proportions of plasma, followed by column

[2] Krebs-Ringer–bicarbonate: 0.118 M NaCl, 0.005 M KCl, 0.0025 M $CaCl_2$, 0.0012 M $MgSO_4$, 0.0012 M KH_2PO_4, 0.025 M $NaHCO_3$, pH 7.4, in equilibrium with 5% CO_2–95% O_2 at 37°C.

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TABLE 1. Composition of activated lymph chylomicrons

% Plasma	TG	CE	FC	PL	PR
0	86.2 ± 1.3	0.7 ± 0.2	1.0 ± 0.2	10.6 ± 0.3	0.7 ± 0.1
20	86.4 ± 2.3	0.7 ± 0.4	1.0 ± 0.1	10.2 ± 0.8	1.1 ± 0.2
40	86.7 ± 1.3	0.7 ± 0.2	1.2 ± 0.1	9.8 ± 1.0	1.5 ± 0.1
60	86.6 ± 1.9	0.7 ± 0.2	0.9 ± 0.2	9.9 ± 1.3	2.0 ± 0.1
80	86.1 ± 1.4	1.0 ± 0.3	1.2 ± 0.2	9.3 ± 1.5	2.1 ± 0.1
100	85.9 ± 1.5	1.1 ± 0.2	1.1 ± 0.2	9.6 ± 1.0	2.2 ± 0.2

Values are means ± SD for three determinations, for chylomicrons (3 mg/ml triglyceride) incubated with plasma for 5 min at 37°C. Composition was determined by standard procedures (see ref. 4) after chromatography using Sepharose 2B.

chromatography, produced particles containing a maximal 3–4-fold increase in protein content, without significant change in the proportions of the major lipid classes (**Table 1**). Protein transfer was complete within 5 min at 37°C. Activation was associated with the incorporation of major amounts of fast-migrating "C-apolipoproteins" and also with that of a slowly migrating component (apo A-1) (**Fig. 1**). Final protein content of chylomicrons incubated with fixed proportions of plasma was dependent on medium TG concentration (**Fig. 2**) but was maximal in 100% plasma for TG concentrations as high as 3 mg/ml medium.

Analysis of the cofactor content of chylomicrons in terms of LPL activation units showed that incubation with plasma was associated with a maximal 4-fold increase in cofactor content (**Fig. 3**). It also indicated a linear relationship between total bound protein and the increase in LPL cofactor content, i.e., there was no change in the proportions of cofactor and added total protein. It is therefore unlikely that concomitant addition of a polypeptide inhibitor of LPL masks a further activation of lipolysis. Since the reaction rate with synthetic triglyceride substrate in the presence of intact or delipidated chylomicrons was the same, the assay was a valid measure of chylomicron content of LPL activator.

In contrast to the major increases induced in chylomicron total protein and LPL cofactor contents, the reaction rate of fully activated chylomicrons with LPL was little different from that of the particles prepared in the absence of plasma protein, and the maximal increase in reaction velocity (1.3-fold) (**Fig. 4**) was obtained in the presence of only 10% plasma protein. Therefore the incorporation of further LPL cofactor, which occurred at higher proportions of plasma (Fig. 3), was not associated with any further increase in the rate of lipolysis. The increase in activation was not significantly different at pH 7.4 and pH 8.3 (1.2 vs 1.3-fold, four experiments).

DISCUSSION

Previous studies from several laboratories have indicated that whereas C-apolipoproteins (which include the cofactor of LPL) were rapidly transferred away to the HDL reservoir in the course of hydrolysis of lipoprotein triglyceride by LPL (5, 6), the catalytic rate with membrane-supported or soluble LPL was maintained until at least 75% of the initial triglyceride content had been catabolized (9). An explanation of this paradox is suggested by the present results, which indicate that a major part of the cofactor protein incorporated with chylomicrons in the presence of whole plasma becomes associated after the cofactor content has ceased to become rate-limiting for LPL activity. Moreover, while this study suggests that as much as half of the chylomicron cofactor content could be removed without effect on the triglyceride hydrolysis rate, this estimate is likely to be minimal, because the very

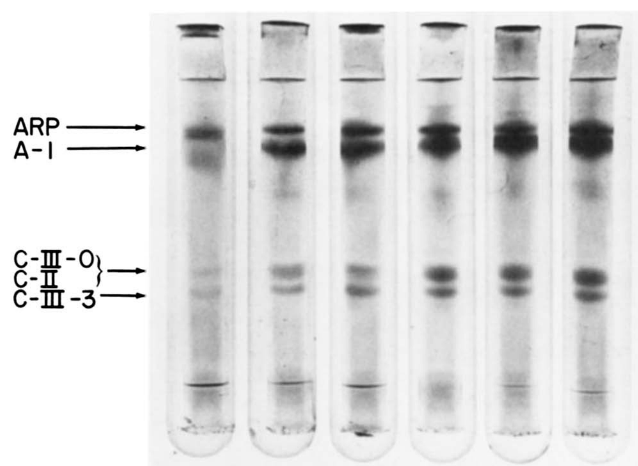

Fig. 1. Electrophoresis patterns of chylomicrons incubated for 5 min at 37°C with (from left) 0%, 20%, 40%, 60%, 80% and 100% plasma protein, and 3 mg/ml TG. Protein added to each gel was that complexed with 4.5 mg chylomicron TG. Component lipoprotein polypeptide species are identified as described by Mjøs et al. (6).

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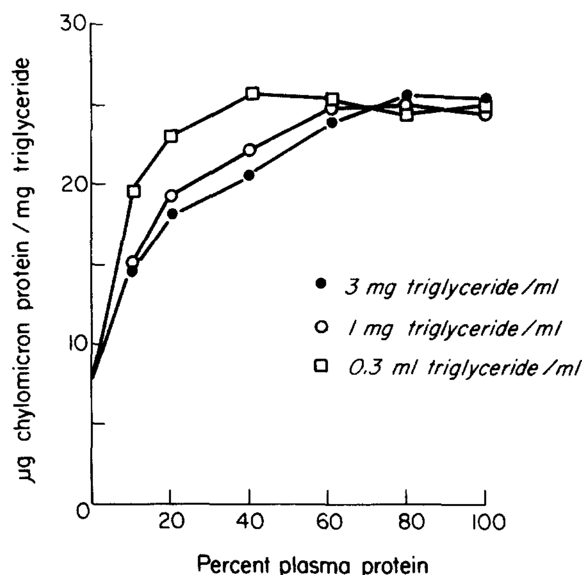

Fig. 2. Protein binding to chylomicrons as a function of medium TG concentration. Incubation conditions as in Fig. 1.

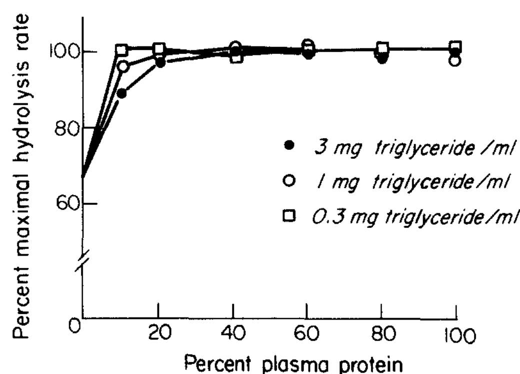

Fig. 4. Activated chylomicrons as substrates for LPL. Assay was for 10 min at 37°C and pH 7.4. Chylomicron TG concentration was 0.25 mg/ml obtained by dilution from solutions of particles incubated with plasma at the proportions shown.

low density lipoprotein fraction, removed from the plasma by the initial centrifugal step, may contain as much as half of the total plasma cofactor protein content, at least part of which is transferrable to triglyceride-rich particles entering the plasma (3). Consequently, even at high plasma triglyceride concentrations, cofactor protein is unlikely to be rate-determining for LPL activity.

Since C-apolipoproteins are not synthesized in the intestinal mucosa (2), the small amounts already present and complexed with intestinal chylomicrons are probably derived from HDL filtered into the lymph. While chylomicrons from intestinal lymph bind considerable additional protein after mixing with the plasma (Fig. 2), including C-apoproteins and apo A-1 (Fig. 1) in particular, this process appears to play little role in increasing the rate of triglyceride catabolism by LPL, at least during hydrolysis of the major part of chylomicron triglyceride (9). Activator content of chylomicrons is therefore not a measure of the hydrolysis rate of lipoprotein triglyceride, at least during the major part of chylomicron catabolism. Regulation of this rate with chylomicrons must lie elsewhere, perhaps in the lipid composition of the lipoprotein particles (12). However, since activator content of chylomicrons is an equilibrium process (Fig. 2), the role of the HDL reservoir of LPL cofactor protein may lie in maintaining maximal hydrolysis rates in the later stages of hydrolysis when small remnant particles that are otherwise poor substrates for this lipase system (9) are involved. This permits the formation of more fully catabolized end products of LPL activity than would otherwise be the case.

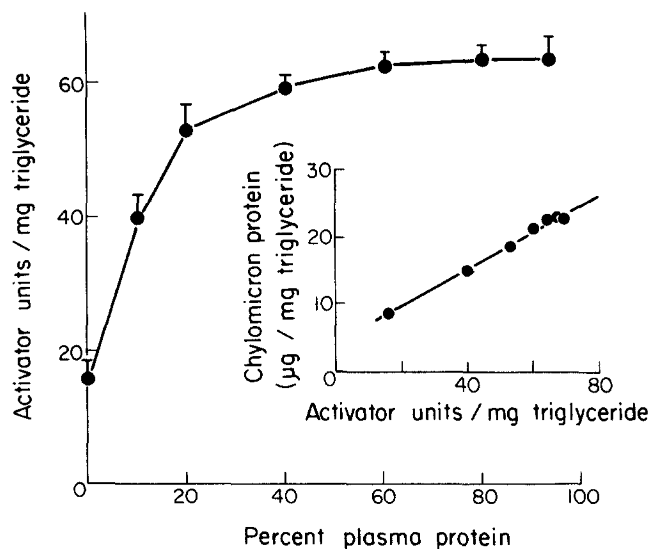

Fig. 3. Activator content of chylomicrons after incubation with plasma. Incubation conditions as in Fig. 1. Activator content was determined as described under Methods for six experimental points in duplicate for each chylomicron preparation.

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